

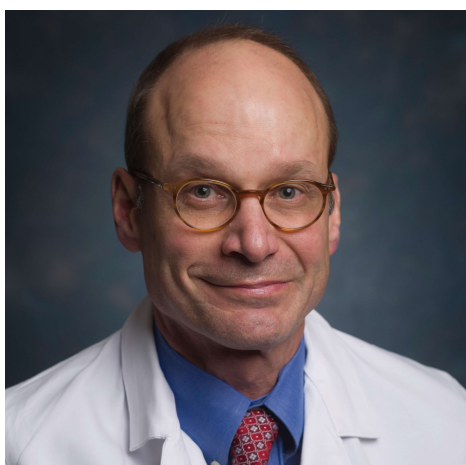
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World Clinical Inflammatory Bowel Disease

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Core progress in clinical management of inflammatory bowel disease in 2014

It is my great pleasure to introduce the first edition of *World Clinical Inflammatory Bowel Disease* (ISBN 978-0-9914430-5-5), the *World* series publication of core progress in clinical management of inflammatory bowel disease, commonly known as IBD. This compendium is published by the Baishideng Publishing Group and comprises the most innovative and significant articles describing clinical and laboratory research published by our journals in 2014. This book is intended to provide a comprehensive summary of our current knowledge on IBDs - from basic pathophysiology to the clinical and bedside practice.

The collected research works and review articles published in 2014 provide an up-to-date overview of the recent progress in our knowledge on the pathogenesis, diagnosis and treatment, clinical management and outcomes in IBDs, highlighting the rapid progression and current challenges in the field. Hot topics of interest include the interplay between microbiota and host factors (*i.e.*, immunopathogenesis, genetics, and intestinal barrier), new data on the changing natural history of adult and pediatric IBDs, objective patient stratification and monitoring, new therapeutic avenues (*e.g.*, stem cell transplantation, Janus kinase inhibitors, and fecal microbiota transplantation), optimization of conventional drug use and current treatment paradigms (*e.g.*, therapeutic drug monitoring of biologicals, pharmacogenetics), special scenarios affecting differential diagnosis (*e.g.*, Bechet's disease and microscopic colitis), and new data on old and rare complications (*e.g.*, primary sclerosing cholangitis, venous thrombosis, neurological/

pulmonary/cardiac complications, and colorectal cancer).

This compendium is composed of 115 articles selected from the *World Journal of Gastroenterology*, *World Journal of Gastrointestinal Pathophysiology*, *World Journal of Gastrointestinal Pharmacology and Therapeutics*, *World Journal of Hepatology*, *World Journal of Gastrointestinal Endoscopy*, and *World Journal of Gastrointestinal Surgery*. The authors represent all continents, making this book truly international and highlighting the modern-day state of IBD research, in its diversity of researchers' backgrounds and expertise, as well as the local clinical challenges.

I hope that this book will prove a useful update to all of our colleagues who are interested in the basic and/or clinical aspects of IBDs, and that it will not only guide future research endeavors but also assist clinicians in their clinical decision making when choosing among the various proven and promising treatment strategies in everyday practice so that the patients' benefit will ultimately be improved.

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Immunopathology of inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) results from a complex series of interactions between susceptibility genes, the environment, and the immune system. The host microbiome, as well as viruses and fungi, play important roles in the development of IBD either by causing inflammation directly or indirectly through an altered immune system. New technologies have allowed researchers to be able to quantify the various components of the microbiome, which will allow for future developments in the etiology of IBD. Various components of the mucosal immune system are implicated in the pathogenesis of IBD and include intestinal epithelial cells, innate lymphoid cells, cells of the innate (macrophages/monocytes, neutrophils, and dendritic cells) and adaptive (T-cells and B-cells) immune system, and their secreted mediators (cytokines and chemokines).

Either a mucosal susceptibility or defect in sampling of gut luminal antigen, possibly through the process of autophagy, leads to activation of innate immune response that may be mediated by enhanced toll-like receptor activity. The antigen presenting cells then mediate the differentiation of naïve T-cells into effector T helper (Th) cells, including Th1, Th2, and Th17, which alter gut homeostasis and lead to IBD. In this review, the effects of these components in the immunopathogenesis of IBD will be discussed.

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Microbiome; Autophagy; T helper 17; Innate immune system; Adaptive immune system; Innate lymphoid cells; TL1A

Core tip: Inflammatory bowel disease (IBD) results from the complex interactions between susceptibility genes, the environment, the immune system, and the host's microbiome. It is thought that either a mucosal susceptibility or a defect in sampling of gut luminal antigen leads to activation of the innate immune system that then recruits cells of the adaptive immune system leading to inflammation. This review will detail the interaction of these components in the immunopathogenesis of IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder that is comprised of both Crohn's

disease (CD) and ulcerative colitis (UC), and is characterized by alternating phases of clinical relapse and remission. CD can affect any part of the gastrointestinal tract and classically presents with fatigue, prolonged diarrhea with or without gross bleeding, abdominal pain, weight loss, and fever. UC characteristically involves the colon and presents with symptoms that usually rectal bleeding, frequent stools, mucus discharge from the rectum, tenesmus, and lower abdominal pain. IBD is thought to be the result of a dysregulated immune system in the context of a genetically susceptible individual. Currently, IBD affects 1.4 million Americans and at a prevalence rate of 396 per hundred thousand individuals worldwide^[1]. The incidence of CD in the United States is estimated to be 5 per hundred thousand persons and is characterized by focal and transmural inflammation that can occur anywhere along the length of the gastrointestinal system, that may include B2 stricturing (gut luminal narrowing), B3 penetrating (bowel perforation, fistula, inflammatory mass/abscess), and with possible perianal disease^[2,3]. UC affects 8-12 per hundred thousand individuals and is characterized by colonic mucosal inflammation along the entire colon and involving the rectum^[3,4]. Also, patients with IBD have an increased risk of developing other chronic inflammatory disorders, such as psoriasis and primary sclerosing cholangitis^[5,6].

The exact cause of IBD is still unknown, but is thought to be due to a combination of a patient's genetics, microbiome, immune response, and the environment that result in an excessive and abnormal immune response against commensal flora in genetically susceptible individuals. Epidemiological data suggest an association between IBD and a number of environmental factors, such as antibiotic use, microbial exposure both early and late in life, and possibly diet^[5,7-9]. The genetics of IBD are complex and thought to be polygenic. Genome-wide association studies (GWAS) suggest that dysregulation in innate and adaptive immunity contribute to the development of IBD. Susceptibility variants have been reported in genes associated with autophagy (*ATG16L1*), the interleukin (IL)-23/Th17 pathway (*IL-12B*), TGF- β pathway (*SMAD3*), T-cell activation (*TAGAP*), among other immune system genes^[10-13].

The identification of these and other loci is only part of a larger picture that aims to understand how polymorphisms in these genes can lead to an increased risk of developing IBD. Here we review the available evidence supporting the role of the microbiome and the innate and adaptive immune responses and their crosstalk in IBD.

THE MICROBIOME

Overview

The interaction of the host with its abundant microbiota is complex. The luminal surface of the small and large intestine, approximately 300-400 m², is a unique environment where an enormous population of bacteria exists

in close proximity to the immune system of the gut mucosa. This roughly translates to the interactions of 10¹² microorganisms per gram of feces with 10⁶ immune cells per gram of enteric tissue^[14]. A complex network of interactions exists between gut epithelial cells, immune cells, and foreign bodies that transition along the gut. Functionally, the gut-associated lymphoid tissues generates either an immune response for rejection of pathogens or a clinical immune response of tolerance for dietary and microbial antigens^[15]. Data supports the hypothesis that IBD results from a dysregulated immune response to the microbiota. It was found that in CD patients, diversion of feces induces inflammatory remission and mucosal healing in the downstream intestinal segment and infusion of feces reactivates the disease^[16]. Furthermore, in UC patients with active disease, treatment with broad-spectrum antibiotics reduced mucosal inflammation^[17]. These data support the concept that luminal bacteria provide the stimulus for an inflammatory response leading to mucosal injury. Two main hypotheses have been suggested that might contribute to the loss of tolerance towards the indigenous microbiota in patients with IBD. First, genetic susceptibility leads to a dysregulation of the mucosal immune system that result in excessive immunologic responses to normal flora. Second, an imbalance exists in the composition of the microbiota that elicits a pathologic response from the normal mucosal immune system^[18]. In all likelihood, it probably is a combination of both hypotheses.

Advancements in genetic technology, such as 16S ribosomal RNA (rRNA) gene and metagenomic sequencing have allowed researchers to determine the composition of the microbiome^[19]. Recently, a number of studies have profiled the "normal" human gut microbiota. Briefly, it is thought that greater than 90% of all phylotypes belong to two divisions, *Bacteroidetes* and *Firmicutes*^[20]. Other divisions that have consistently been recovered from "normal" individuals include *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia*. It is believed that the composition of fecal microbiota remains relatively constant over time, termed resilience, with temporary changes occurring after exposure to food, medicine, and physical environment^[21]. In 2010, whole-genome shotgun sequencing revealed 3.3 million nonredundant microbial genes in fecal samples from an adult European cohort^[22]. It was found that up to 98% of the genes were bacterial with the rest belonging to archaea, yeasts, viruses, and protists. The three most abundant genera in the fecal samples were *Bacteroides*, *Faecalibacterium*, and *Bifidobacterium*, however the percent composition was found to be highly variable between individuals^[23]. Three main enterotypes (independent of gender, age, race, body mass index, or country and continent of residence) were created that are classified based upon the prominent genera represented: enterotype 1-*Bacteriodes*; enterotype 2-*Prevotella*; enterotype 3-*Ruminococcus*^[23].

Individuals with IBD have been shown to have changes in the bacterial composition of feces with less bacterial

diversity, having fewer numbers of non-redundant bacterial genes, as compared to healthy controls^[24,25]. Adding to the complexity, the bacterial profile also differs between individuals with UC and CD. In a twin study, twins with UC were found to have less *Bacteroides* and more *Actinobacteria* and *Proteobacteria* than their healthy twin counterparts. The decrease in *Bacteroides* was made up for by an increase in the Prevotellaceae family^[26]. Also, it has been found that *Escherichia coli* (*E. coli*) is to be increased in fecal samples from individuals with UC, with some isolates expressing virulence factors and invading properties^[27,28]. Dysbiosis also exists in CD. Many studies have shown a decrease in the abundance of several bacterial species of the phylum *Firmicutes* in patients with CD^[29,30]. Also, the microbiome of individuals with CD predominantly in the ileum was found to differ from those whose disease was found predominantly in the colon. Those with ileal CD had decreased *Faecalibacterium* and *Roseburia* and increased amounts of *Enterobacteriaceae* and *Ruminococcus gnavus*^[31]. Consistent with what is seen in UC patients, *E. coli* also has been observed in patients with CD^[27].

Recently a new pathogenic group called adherent-invasive *E. coli* (AIEC) has been isolated from the ileum of CD patients^[30,32]. Darfeuille-Michaud^[33] have accumulated a large body of data showing that AIEC is able to invade epithelial cells and to survive and replicate within macrophages and is associated with ileal mucosal CD pathogenesis. They found that AIEC was present in the inflamed ileum of 22% of chronic CD patients, as compared to only 6% of control patients, as well as 36% of the newly formed terminal ilea of postsurgical CD patients^[34]. However, AIEC was found in only a small percentage of affected colons of CD patients and in zero percent of UC patients, suggesting that AIEC strains are associated specifically with ileal mucosa in CD^[34]. AIEC facilitate binding and invasion into epithelial cells *via* type 1 pili and flagella^[35]. This interaction is dependent upon epithelial expression of CEACAM6, which is a carcinoembryonic antigen upregulated by inflammatory cytokines and possibly by AIEC itself^[36]. Furthermore, transgenic mice overexpressing CEACAM6 in epithelial cells are colonized by AIEC and manifest gut inflammation with marked neutrophil infiltration and ulceration^[37]. In the lamina propria, AIEC is taken up by macrophages and can survive and proliferate within macrophage vacuoles, which suggest that the bacteria is not readily cleared from the site and may represent a defect in autophagy^[38,39].

Also, recently an important role of fungi in gut homeostasis has been found. Besides the massive amount of bacteria known to make up the intestinal microflora, the mammalian gut also contains a rich fungal community that interacts with the immune system *via* Dectin-1, which is a pattern-recognition receptor expressed by innate immune cells, such as: macrophages, dendritic cells (DCs), and neutrophils^[40]. It was found that mice deficient in Dectin-1 exhibited increased susceptibility to dextran sulfate sodium (DSS) colitis that was the result of an

altered response to the host's fungi^[41]. Furthermore, polymorphisms in the gene for Dectin-1 have been shown to be strongly linked to a severe form of UC^[41]. This data suggests that the interactions between fungi and the innate immune system are important in the development of IBD. Viral infections also impact the gut microflora. In mice, virus-plus-susceptibility gene interactions have been shown to induce colitis that mimics CD. When mice with a specific mutation in a CD susceptibility gene for autophagy (*ATG16L1*) were infected with murine norovirus they displayed abnormal Paneth cell structure and granule packaging similar to those seen in CD patients homozygous for the risk allele of *ATG16L1*^[42]. These changes were not seen in control mice nor in CD patients homozygous for the nonrisk allele of *ATG16L1*. Furthermore, when these mice were treated with DSS they displayed worse colitis than control animals^[42]. These results demonstrate how a genetic factor and an environmental agent can contribute to the pathogenesis of CD. Also, HIV infection of humans and simian immunodeficiency virus (SIV) infection of rhesus monkeys is known to cause systemic immune activation and associated with damage to the intestinal epithelium and translocation of antigens into the blood^[43-45]. Pathogenic SIV infection has been associated with significant expansion of the enteric virome, including adenovirus and parvovirus, that can lead to enteritis without changes in the microbiome^[46]. This data suggests that the enteric virome might contribute to AIDS pathogenesis by damaging the intestinal epithelium to allow translocation of microbes and viral antigens into the circulation.

The consequences of these shifts in microbiota are unclear, particularly whether it is cause or effect. Regardless of the inciting cause of IBD, it is apparent that the host-microbiome interaction plays a large part in disease pathogenesis.

Host-microbial interactions

Host-microbe interactions are crucial in the development and modulation of the immune system and protection from pathogenic bacterial invasion. The first line of defense to pathogenic organisms is the innate immune system, which in the gut consists of mucin, the epithelium, and cells of the innate immune system [*i.e.*, neutrophils, DCs, monocytes/macrophages, and innate lymphoid cells (ILCs)]. Interestingly, mice lacking an adaptive immune system, but that have an intact innate immune system, such as recombination activation gene deficient (*RAG*^{-/-}) and severe combined immunodeficient (SCID) mice, do not develop spontaneous colitis and co-exist with the microbiota. However, these mice can develop colitis when induced by DSS, anti-CD40 antibody, and *Helicobacter hepaticus* infection^[47-49]. These data suggest that in the absence of an adaptive immune system, the innate immune system is sufficient for the development of IBD. However, the adaptive immune system is still thought to play an important role in the development of IBD, as *RAG* deficiency can prevent the development of spontaneous

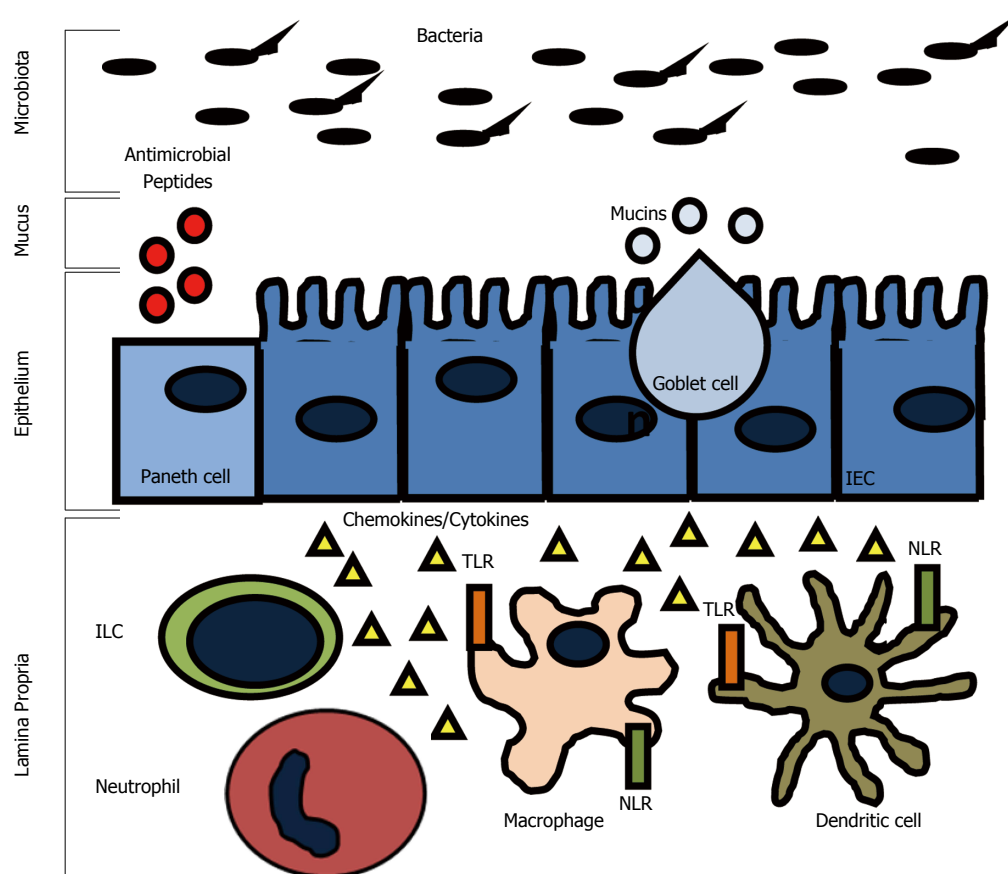


Figure 1 Innate immune system responses in the gut. The intestinal epithelial barrier is equipped with several layers of defense mechanisms to limit luminal antigen translocation. Goblet cells, Paneth cells, and enterocytes secrete mucins and antimicrobial peptides that assemble into a mucus layer. Innate immune system cells, such as macrophages and dendritic cells, can sense invading bacteria through extracellular and intracellular pattern recognition receptors (Toll-like receptors-TLRs and NOD-like receptors-NLRs) and initiate rapid inflammatory responses mediated by the secretion of cytokines and chemokines. Innate lymphoid cells (ILCs) are also found in the lamina propria where they contribute to cytokine production and inflammatory cell recruitment.

colitis that are seen with certain mutant mouse strains^[50].

INNATE BARRIERS OF PROTECTION

Mucus

The surface of the intestine is protected by a layer of mucus that is generated by goblet cells in the epithelium (Figure 1). The inner mucus layer is approximately 100 μm thick, firmly adherent, rich in antimicrobials and mucin, and has a low bacterial density. The outer layer of mucus is comprised of mucin, diluted antimicrobials, and some bacteria. A variant in the *Muc2* gene, which is the major intestinal secretory mucin, confers susceptibility in humans to IBD and *Muc2* deficient mice develop spontaneous colitis^[51]. Furthermore, some patients with CD have been found to have goblet cell depletion and an impaired mucus layer, which allows bacteria to adhere directly to epithelial cells, and may contribute to disease progression^[52]. It is believed that the ability of commensal bacteria to adhere to the epithelial layer *via* oligosaccharides helps deter invasion by displacing pathogenic bacteria. *FUT2* is a gene that encodes a type alpha (1, 2) fucosyltransferase, which regulates the secretion of the H1 antigen of the ABO antigens into the mucosa. People are either associated as H1 antigen secretors or non-

secretors. Twenty percent of the population are non-secretors, which has been associated with a variety of illnesses including recurrent norovirus and encapsulated bacterial infections, duodenal ulcerations, and susceptibility to CD^[53-59]. The inability to secrete H1 into the mucosa is thought to affect how commensal and pathogenic flora interact with the epithelial layer and may interfere with the ability of the commensal flora to adhere, which could result in increased susceptibility to infection, invasion, and activation of the immune system.

Epithelium

The epithelium of the intestine has many functions, including absorption, secretion, and digestion. There are four main types of epithelial cells: one, absorptive enterocytes; two, mucus producing goblet cells; three, hormone producing enteroendocrine cells; and four, antimicrobial and growth factor producing Paneth cells (Figure 1). The epithelium forms a mucosal barrier with tight junctions between enterocytes that can exclude the entry of most substances. The epithelial layer is renewed every 2-3 d with a balance of proliferation of epithelial cells in the crypts and migration down the villi in the small intestine or onto the surface of the colon and apoptosis and shedding of the enterocytes. Disruption of this process im-

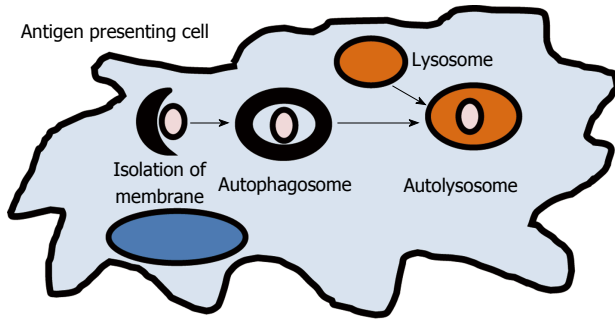


Figure 2 Autophagy. A small volume of cytoplasm is enclosed by the autophagic isolation membrane, which forms the autophagosome. The outer membrane of the autophagosome then fuses with the lysosome where the cytoplasm derived materials are degraded.

pairs the epithelial integrity and can result in chronic inflammation. Defects in epithelial integrity may contribute to IBD pathogenesis by allowing free passage of organisms across the epithelial layer where they can incite an immune response. For example, mutations in the *organic cation transporter* (*OCTN*) gene, which is involved in the transport of cationic proteins, such as amino acids and nutrients, lead to an increased susceptibility to CD^[60]. The susceptibility is thought to result from impaired fatty acid oxidation, which can cause colitis in experimental models in the setting of bacterial antigen exposure^[61].

The epithelium lies between the immune cells in the lamina propria and the microbiota in the gut lumen and functions to communicate with both. For example, the microbiota signals enterocytes as well as innate cells in the lamina propria *via* pattern recognition molecules signal receptors, such as toll like receptors (TLRs) and cytosolic NOD-like receptors (NLRs) (Figure 1). These signals have been shown to be necessary for normal homeostasis and resistance to injury^[62]. Cytokines, such as interferon (IFN)- γ , interleukin (IL)-17, and IL-22, pathogens, and commensal bacteria have substantial effects on the epithelium by regulating barrier integrity and function^[63-66]. Expression of pattern recognition receptors is highly regulated to prevent an inappropriate immune response, but still allow for constant surveillance. Mutations in genes coding for these receptors have been found to be IBD susceptibility genes. Haplotypes of the *TLR8* gene can confer protection (H1) or risk (H4) to the development of IBD^[67]. Agonists of *TLR8* have been shown to cause downstream activation of proinflammatory cytokines such as IFN- γ , IL-12, and tumor necrosis factor (TNF)- α in peripheral blood mononuclear cells^[68]. The *TLR9* gene is also a CD and UC susceptibility gene^[69]. It has been shown that mice infected with *Campylobacter jejuni* (*C. jejuni*) or *TLR9* agonists have increased susceptibility to mild DSS colitis *via* a mechanism involving secretion of CXCL8^[70].

Defects in the intestinal barrier can lead to persistent immune activation and has been suggested to play a role in IBD^[71]. Normally, translocation allows small amounts of luminal antigens to pass transcellularly across the epithelium either through receptor-mediated endocytosis or

non-selective endocytosis. A small amount of bacteria is normally allowed to translocate and allows for physiologic sampling of luminal content by the host's immune system^[72]. Animal models that lack components of a healthy epithelial barrier have been shown to develop IBD. Expression of dominant-negative N-cadherin in mouse intestinal epithelium has been shown to lead to CD like symptoms^[73]. NOD2 is a protein that acts as an intracellular pattern recognition receptor for muramyl dipeptide (MDP), a component of the bacterial wall peptidoglycans. Mice lacking intracellular pattern recognition receptors, NOD1 and NOD2, were shown to have decreased E-cadherin expression with increased epithelial permeability and decreased antimicrobial production^[74]. *NOD2* was one of the first CD susceptibility genes, with homozygous mutations found in 15% of patients with CD^[75]. Mutations in *NOD2*, as well as other pattern recognition receptors, might impair the ability of the mucosal immune system to sense organisms thereby leading to defective microbial clearance and persistent antigenic stimulation. This in turn may result in mucosal inflammation and loss of regulatory control over proinflammatory pathways, which could possibly lead to the development of IBD.

AUTOPHAGY

The term autophagy, or “self-eating,” results in the lysosomal degradation of organelles, unfolded proteins, or foreign extracellular material (Figure 2). It is a key process required for maintaining cellular homeostasis after infection, mitochondrial damage, or ER stress. Defects in autophagy have been shown to result in pathological inflammation and GWAS have linked two key genes in autophagy, *ATG16L1* and *IRGM*, to CD^[13,76]. An *ATG16L1* hypomorphic mouse line that expresses about 1% of the normal level of *ATG16L1* was shown to have Paneth cell granule abnormalities that are similar to those found in ileal resections in patients with CD that also carry the *ATG16L1* gene variant^[77]. While these hypomorphic *ATG16L1* mice do not develop spontaneous colitis, they were found to have an increased susceptibility to DSS colitis^[42]. However, when rederived virus free, these mice lost the Paneth cell pathology and ability to develop DSS induced colitis, which could be reversed by norovirus infection^[42]. A recent study has reported that the *ATG16L1* and *NOD2* pathways may be interrelated^[78]. In 2010, Cooney *et al.*^[78] demonstrated that *NOD2* stimulation is capable of initiating autophagy in DCs and that for effective autophagy to occur, both intact *NOD2* and *ATG16L1* functions are required. *IRGM* belongs to a family of interferon-inducible immunity related GTPases (IRGs) that encodes a protein involved in multiple autophagocytic pathways including intracellular clearance of pathogens^[79]. *IRGM* has been shown to play a role in autophagy during both *Salmonella typhimurium* and *Mycobacterium bovis* infections^[79,80]. Another study in CD patients has demonstrated that autophagy is also important in the clearance of AIEC and that *IRGM* and *ATG16L1*

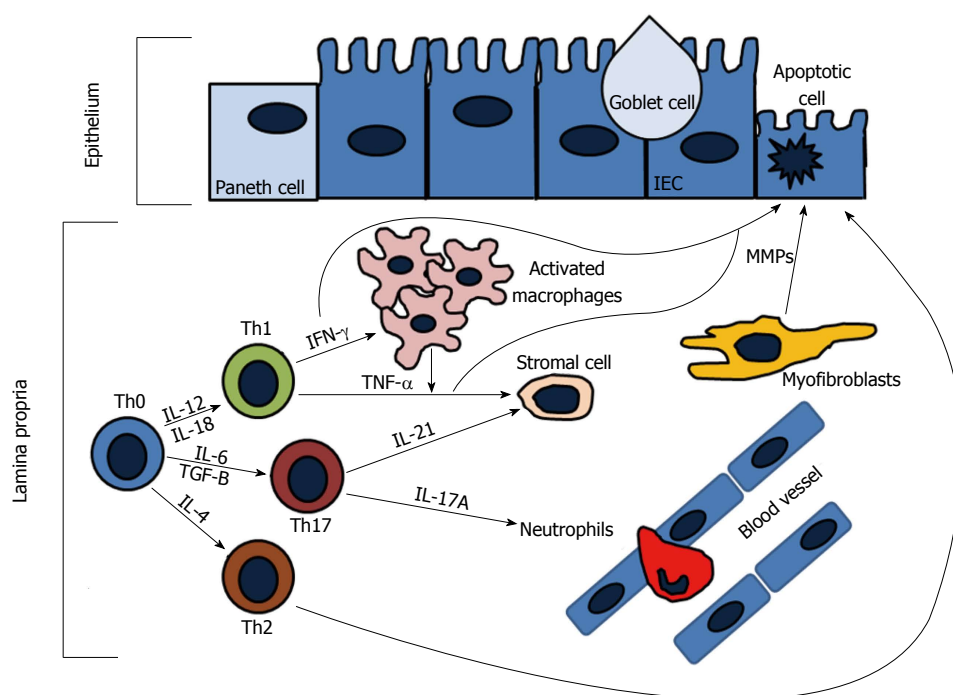


Figure 3 Adaptive immune responses in the gut. During active inflammation, naïve T-cells (Th0) differentiate into T helper cell types (Th1, Th2, Th17) under stimulation of different cytokines. Th1 cells produce interferon (IFN)- γ and tumor necrosis factor (TNF)- α . IFN- γ activated tissue macrophages to produce additional TNF- α , which causes epithelial cell apoptosis and differentiation of stromal cells into myofibroblasts. Activated myofibroblasts produce metalloproteinases (MMPs) that cause tissue degradation. Th2 cells produce interleukin (IL)-13 that can increase intestinal permeability and induce epithelial apoptosis. Th17 cells release IL-17A, which plays a role in recruiting neutrophils to sites of active inflammation, and IL-21 that also induces MMP production that contributes to extracellular matrix degradation.

deficient cells had increased AIEC replication, suggesting that these genes play a significant role in clearance of this organism and intestinal inflammation^[39]. These studies implicate that autophagy plays an important role in human inflammatory disorders by direct elimination of intracellular bacteria and activation of pattern recognition receptor signaling which is involved in gut homeostasis and CD pathogenesis.

INNATE AND ADAPTIVE IMMUNITY

Overview

The immune system has evolved as protection against a wide range of infectious agents. In vertebrates, the immune system is broadly divided into two effector classes, the innate and adaptive immune responses. The innate immune system is the first line of defense and provides an immediate protective response against infections and also helps to initiate the adaptive immune response (Figure 1). The innate immune system is non-specific and does not confer lasting immunity (memory). The innate immune system is comprised of the epithelial barrier, macrophages, monocytes, neutrophils, DCs, and natural killer cells (NK cells), eosinophils, and basophils. These cells act together to initiate inflammation by secreting cytokines, chemokines, and antimicrobial agents. This leads to phagocytosis of infected cells and microorganisms, antigen presentation, and activation of the adaptive immune system.

The adaptive immune response is comprised of lym-

phocytes (T and B cells) that when activated generate effector responses (cytokines and antibodies). In contrast to the innate immune system, the adaptive immune system is highly specific and confers long lasting immunity (memory). It is generally thought that the adaptive immune system is the main contributor to disease pathogenesis in IBD, either through increased proinflammatory cytokines driven by the T-helper (Th) subsets or by ineffective anti-inflammatory regulatory T-cells (Tregs). Naïve T-cells (Th0) cells after activation are able to differentiate into Th1, Th2, or Th17 cells (Figure 3). In particular, Th1 responses have been thought to drive the pathogenesis of CD, while UC is thought to be driven by Th2 responses. Recent advancements suggest that other cells, such as ILCs and Th17 cells, have emerged as important contributors to IBD pathogenesis.

Role of innate lymphoid cells in IBD

Until recently NK cells were thought to be the only innate cell derived from a lymphoid progenitor. However, recent developments have classified NK cells as a subset of a new family of hematopoietic effector cells called ILCs. ILCs are an emerging and diverse group of immune cells and are part of the new frontier of immunology research. All ILCs derive from an Id2 expressing progenitor and are defined by three main features: One, they are of lymphoid morphology; two, they are cell lineage negative (CD3⁻, B220⁻, GR1⁻, CD11b⁻, Ter119⁻); and three, they lack RAG-dependent antigen receptors (Figure 4)^[81]. Recently, a unifying ILC classification system has

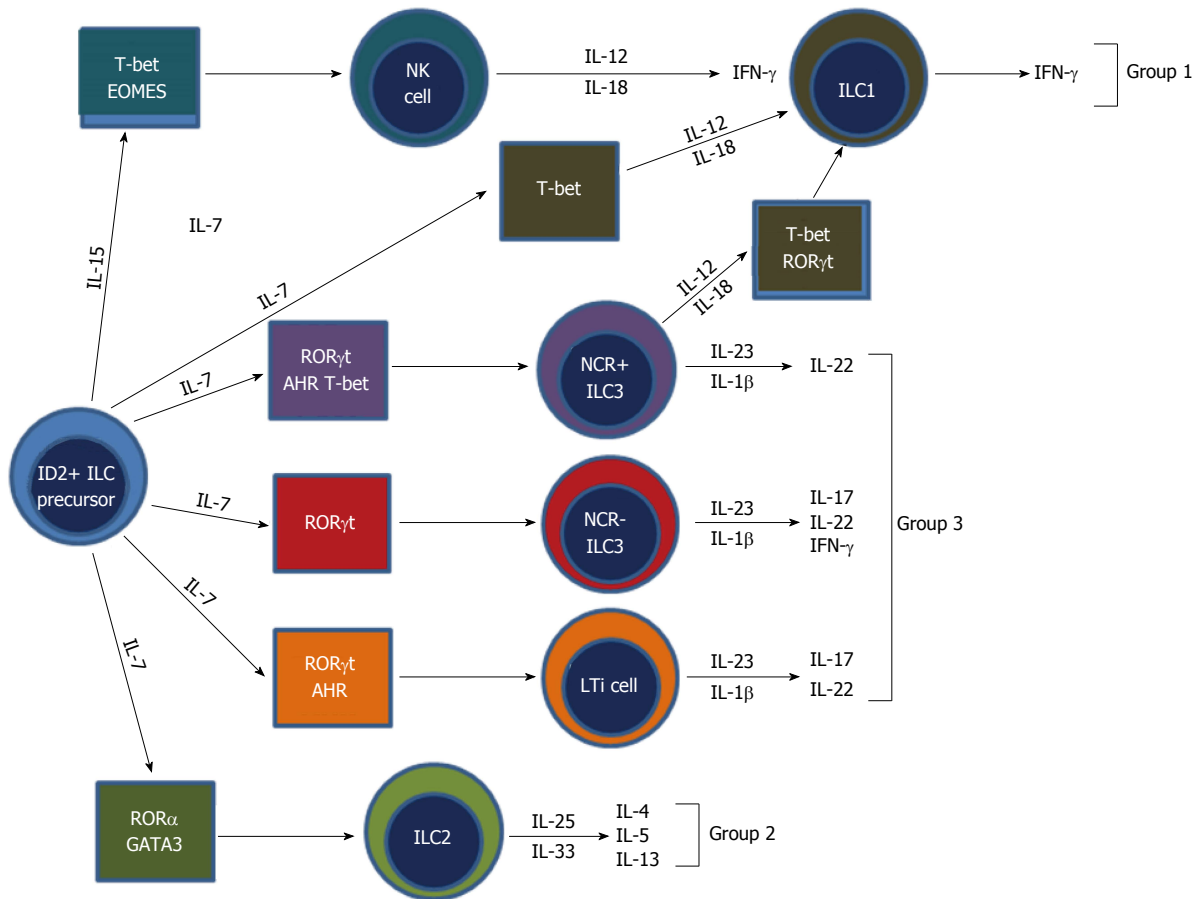


Figure 4 Development and classification of innate lymphoid cells^[82]. Innate lymphoid cells (ILCs) all derive from an ID2 positive progenitor cell. Group 1 ILCs make interferon (IFN)- γ . Group 2 ILCs produce IL-5 and interleukin (IL)-13. Group 3 ILCs produce IL-17, IL-22, and IFN- γ . NK require IL-15, whereas all other ILCs require IL-7 for development. Group 2 ILCs depend on transcription factors GATA3 and ROR for development. Group 3 ILCs require ROR γ t for development. Also, subsets of group 3 ILCs require additional transcription factors, such as aryl hydrocarbon receptor (AHR) for development. NK cells, which are group 1 ILCs, require both T-bet and eomesodermin (EOMES). The mechanisms of ILC1 development are not fully elucidated, however are known to require transcription factor T-bet for development.

based upon phenotypic and functional characteristics has been proposed (Table 1)^[82]. ILCs can be classified into three groups: Group 1 ILCs, which are T-box expressed in T-cells (Tbet) dependent and are comprised of ILC1 and NK cells; group 2 ILCs, which are GATA-binding protein 3 (GATA3) and retinoic acid receptor-related orphan receptor (ROR) dependent, and comprised of ILC2s; and group 3 ILCs, which are ROR γ t dependent and are comprised of ILC3s and lymphoid tissue-inducer (LTI) cells^[82].

The key cytokines secreted by ILCs tend to mirror those secreted by the T-helper cells of the adaptive immune system and therefore ILCs have been thought of as the innate counterparts of T-helper lymphocytes (Table 1). Group 1 ILCs are defined by their ability to produce Th1 cell associated cytokines, in particular IFN- γ . Although under debate, the prototypical cell is the NK cell. Group 2 ILCs are defined by their ability to produce Th2 cytokines, in particular IL-5 and IL-13 and the prototypical cells are the IH2 cells or nuocytes^[83-86]. These cells have been shown to play a major role in defense against parasites and in allergy and asthma^[87,88]. Group 3 ILCs are defined by their ability to secrete Th17 like cytokines

such as IL-17 and IL-22 and the prototypical cells are LTI cells^[89]. Group 3 ILCs have been shown to play a major role in autoimmune disease and have been shown to mediate colitis in a mouse model of IBD^[47].

Since ILCs have been shown to be important in mucosal immunity it was only logical to examine the role of these cells in IBD. Recent data has implicated ILCs, in particular group 3 ILCs in the development of IBD. While most research into IBD has focused on the role of the adaptive immune system, in particular Th1 and Th17 subsets, as well as ineffective regulatory T-cells, new evidence suggests that IBD can be triggered in RAG^{-/-} mice, which lack all components of the adaptive immune system in an IL-23 dependent manner. Buonocore *et al.*^[47] demonstrated that group 3 ILCs, and not NK cells (group 1 ILCs), were increased and produced large amounts of IL-17A and IFN- γ after *Hepaticus* infection in RAG^{-/-} mice were required for colitogenesis. Accumulating evidence suggests that group 3 ILCs induce colitis *via* an IL-23R-IL-22 dependent mechanism^[47,90,91]. RAG^{-/-} mice can also develop colitis after injection of CD40L. Vonnahme *et al.*^[92] demonstrated that CD40L induced colitis requires the presence of innate lymphocytes because CD40L

Table 1 Innate lymphoid cell subsets

ILCs	Lineage	Mouse	Human	Cytokines	Function	Disease
Group 1	ILC1s	Lin ⁻ Thy1 ⁺ Sca1 ⁺ Tbet ⁺	Lin ⁻ CD56 ⁺ NKp46 ⁺ NKp30 ⁺ NKp44 ⁺ IL-7R ⁺	IFN- γ	Inflammation	IBD?
	NK cells	NKp46 ⁺ NK1.1 ⁺ CD122 ⁺ NKG2D ⁺ CD161 ⁺ CD16 ⁺	CD122 ⁺ NKG2D ⁺ CD161 ⁺ KIR ⁺	IFN γ , TNF- α , cytotoxic effectors	Immunity to viruses and intracellular pathogens, tumor surveillance	Inflammatory conditions, IBD
Group 2	ILC2s	Lin ⁻ ICOS ⁺ Thy1 ⁺ Sca1 ⁺ IL-7R ⁺ GATA3 ⁺	Lin ⁻ IL-7R ⁺ CD45 ^{hi} CD161 ⁺ CRTH2 ⁺	IL-5, IL-9, IL-13	Immunity to helminthes, wound healing	Allergy, asthma
Group 3	ILC3s	Lin ⁻ Thy1 ⁺ Sca1 ⁺ RORt ⁺ NKp46 ⁺ IL-7R ⁺ CCR6 ⁺	Lin ⁻ CD56 ⁺ NKp46 ⁺ NKp30 ⁺ NKp44 ⁺ IL-7R ⁺	IL-22	Lymphoid tissue development, intestinal homeostasis, immunity to extracellular bacteria	IBD
	LTi cells	Lin ⁻ Thy1 ⁺ Sca1 ⁺ RORt ⁺ NKp46 ⁺ IL-7R ⁺ CCR6 ⁺	Lin ⁻ IL-7R ⁺ CD45 ⁺ RORt ⁺	IL-17A, IL-17F, IL-22	Homeostasis of epithelia, immunity to extracellular bacteria	IBD

Lin⁻: Lineage marker negative (mouse negative for CD3, CD19, B220, CD11b, CD11c, GR1, Ter11; human negative for CD1a, CD3, CD11c, CD34, CD123, TCR, CD19, CD14, CD16); IBD: Inflammatory bowel disease; ILC: Innate lymphoid cell; IFN: interferon; TNF: Tumor necrosis factor; NK: Natural killer.

injection into RAG2^{-/-}-IL-2Rg^{-/-} mice, which lack both adaptive immune cells and ILCs, did not develop colitis. Furthermore, group 3 ILC involvement in IBD has been further supported by the observation that RORt^{-/-} mice do not develop CD40L induced colitis^[47]. In an elegant set of experiments, it was found that LTi cells were the subset of group 3 ILCs that were colitogenic and required for disease onset, suggesting an important role for these cells in IBD pathogenesis^[47]. In individuals with CD, a population of innate lymphocytes that were RORt⁺ and represent human ILCs were found to be increased in the lamina propria compared to controls and this increase was IL-23 dependent^[93,94]. Although a young field, data suggests that group 3 ILCs are an important cell type to study for answering questions about the pathogenesis of IBD as well as possible future therapies.

Th1 and Th2 cells

Th1 cells are induced by IL-12 and characteristically secrete copious amounts of IFN- γ , TNF- α , and IL-12, whereas the signature cytokines secreted from Th2 cells are IL-4, IL-5, and IL-13^[95]. CD is thought to be a Th1 mediated disease, while UC is believed to be mediated by Th2 responses^[96]. Mucosal T-cells from CD patients have been shown to secrete higher amounts of IFN- γ and IL-2 than from T-cells from UC patients^[97,98]. Furthermore, it has been demonstrated that UC patients produce increased amounts of IL-5 and have atypical natural killer T (NKT)-cells that secrete higher amounts of IL-13 as compared to CD patients^[99-101]. However, recent data has suggested that the CD-Th1 and UC-Th2 paradigms are not so straight forward. Biopsies from both CD and UC patients have demonstrated high *ex vivo* levels of IFN- γ and lower levels of IL-13 have been found in UC patients as compared to CD patients^[102,103]. Furthermore, data suggests that Th17 cell production of IL-17 and IL-23 play important roles in the pathogenesis of IBD, with DCs isolated from CD patients producing more IL-23 than UC patients^[104]. Understanding the complicated interactions underlying the dysregulated adaptive immune response in IBD will ultimately identify novel therapeutic

targets.

Th17 cells: Friend or foe

Th17 cells are a subset of helper T-cells that are induced by IL-6 and TGF- β , expanded by IL-23, and characterized by the secretion of copious amounts of IL-17A, IL-17F, IL-21, and IL-22^[105-107]. RORt has been identified as the master transcription factor of Th17 differentiation^[108,109]. The IL-17 cytokine family includes six members: IL-17A-F^[110]. IL-17A and IL-17F are 50% similar in their amino acid structure, while IL-17B, IL-17C, and IL-17D have less homology^[111]. IL-17A and IL-17F signal through the same receptor, the IL-17 receptor A (IL-17RA) and act through activation of the NF- κ B and MAPK pathways^[112,113]. The major proinflammatory effects of IL-17A and IL-17F are the activation of various cellular targets, including the epithelium, endothelium, monocytes/macrophages, fibroblasts, and neutrophils that cause the induction of TNF- α , IL-1 β , chemokines (CXCL8, CXCL9, CXCL10), GM-CSF, G-CSF, IL-6, and metalloproteases^[114-117]. There are two major subsets of Th17 cells: Th17 cells producing IL-17 and Th1/Th17 cells producing both IFN- γ and IL-17^[104,118-122]. IL-17 has been implicated in various immune mediated diseases, including rheumatoid arthritis (RA), asthma, IBD, and experimental autoimmune encephalitis (EAE)^[123,124].

Th17 cells and signature cytokines have been extensively studied in IBD. GWAS have identified several genes involved in Th17 differentiation and expansion, including *IL-23R*, *IL-12B*, *JAK2*, *STAT3*, *CCR6* and *TNFSF15*, as CD susceptibility genes with some overlap in UC^[12,58]. As compared to normal, CD and UC patients have increased levels of IL-17A gut mucosal transcripts and the lamina propria contains increased numbers of Th17 and Th1/Th17 cells^[102,123,125]. RORt is found to be expressed at higher levels in lamina propria T-cells from CD patients^[126].

Th17 pathobiology is complicated by the fact that in different experimental models, Th17 subsets can be distinguished by their function as either “pathogenic” or “nonpathogenic”. Pathogenic Th17 cells are thought

to be characterized by their production of IFN- γ and by the expression of specific surface markers, including IL-18R1 and CXCR3^[127]. IL-17A deficient mice or those treated with neutralizing antibodies to IL-17A or IL-17RA are resistant to the development of RA and EAE^[128,129]. Furthermore, in a trinitrobenzene sulfonic acid (TNBS) mouse model of colitis IL-17RA deficient animals were protected from the development of acute mucosal inflammation^[130]. However, in a DSS model of colitis, mucosal inflammation was ameliorated by IL-17F deficiency, but exacerbated by IL-17A deficiency, suggesting an important role for IL-17F and perhaps an alternative role for IL-17A^[131-133]. Furthermore, supporting a protective role for IL-17A, it has been shown that IL-17A directly inhibits Th1 cells and suppresses development of inflammation^[134]. Additionally, anti-IL-17A monoclonal antibody treatment was shown to exacerbate DSS induced colitis^[135]. These studies suggest that IL-17A may protect against the development of mucosal inflammation whereas IL-17F may drive it.

As demonstrated by the data above, the biology of IL-17 deficiency has been complicated, with some studies showing a pathogenic role, while others suggesting a protective role. However, there has been a significant amount of data suggesting that IL-17A has played a pathogenic role in IBD. Therefore, a double-blind, randomized, placebo-controlled study tested whether the anti-IL-17A monoclonal antibody, secukinumab, would be beneficial in CD patients^[136]. Surprisingly, the study found that blockade of IL-17A was ineffective and caused a higher rate of adverse events as compared to placebo, suggesting a protective role of IL-17A^[136]. Although the study was halted prematurely, exploratory analysis of CD candidate genetic polymorphisms found that a subset of patients with a minor allele of TL1A actually had an improved clinical score over the course of the treatment^[136]. These data suggest that in the right genetic context, secukinumab therapy may be beneficial to some patients, which further supports the concept of treating each individual with IBD based upon their own genetic composition.

TL1A: CONNECTING THE INNATE AND ADAPTIVE IMMUNE SYSTEM

Tumor necrosis factor super family 15 (*TNFSF15*) encodes the protein TL1A, a member of the TNF superfamily, is expressed either membrane bound or secreted by monocytes, macrophages, DCs, fibroblasts, and endothelial cells in response to stimulation by cytokines and microorganisms^[137-140]. It binds to death domain receptor 3 (DR3), mainly expressed on T-cells, to initiate a number of immune responses, such as activation of T-cells resulting in the secretion of proinflammatory mediators^[141]. TL1A has been implicated in the pathogenesis of many autoimmune diseases, including asthma, rheumatoid arthritis, and IBD^[96,140,142-144]. Numerous studies have supported the concept that TL1A is a major regulator of mucosal

inflammation at the interface between the innate and adaptive immune system^[145-147].

In 2005, in a study of Japanese CD patients, polymorphisms in the *TNFSF15* gene was identified as having a strong association with CD^[148]. This association has been reproduced in other studies, including European and Jewish CD and UC patients, and has been demonstrated to be the dominant gene in East-Asians with IBD^[12,149-153]. Haplotypes within the gene confer either risk or protection, which is dependent upon the ethnicity of the individual. In non-Jewish CD patients, haplotype A is a risk allele, while haplotype B is protective^[148-150,153]. However, in Jewish CD patients, haplotype B has a trend towards risk, as these patients had worsened disease as manifested by higher incidents of surgery and increased responses to *E. coli* outer membrane porin C (OMPC)^[154,155]. Furthermore, monocytes isolated from Jewish patients that were haplotype B secreted increased amounts of TL1A than haplotype A carriers after stimulation^[156].

Given the information generated from the human GWAS studies, transgenic mice have been created that overexpress TL1A. In 2011, Meylan *et al.*^[146] and Taraban *et al.*^[147] found that murine colitis driven by TL1A overexpression in T-cells and DCs was found to be dominated by a Th2 response over Th1, with elevation in IL-13 and unchanged levels of IFN- γ . Also, in both models, spontaneous intestinal inflammation developed, with disease severity being greatest in the terminal ileum and correlating to transgene expression level. This observation was abolished with anti-IL-13 treatment. Shih *et al.*^[143] reported similar observations in another model of TL1A overexpression. However, they also found that these mice had increased levels of IFN- γ and intestinal fibrosis. The differences in these mouse models may be secondary to differences in the generation of the mice and/or different gut microflora between animal facilities. Regardless, all models demonstrated intestinal inflammation and this supports evidence of TL1A polymorphisms being associated with IBD. Given this information, studies utilizing anti-TL1A antibodies were undertaken. In models of TNBS and DSS colitis anti-TL1A neutralizing antibody treatment was shown to ameliorate weight loss and intestinal inflammation^[146,157]. These studies suggest a role for using blocking antibodies to TL1A to ameliorate pathological T-cell responses in IBD.

CONCLUSION

In current immunology there are new Th cell subsets, such as IL-9 producing Th9 cells, IL-22 producing Th22 cells, follicular helper T-cells, and emerging types of Treg cells that are now also all being implicated in the pathogenesis of IBD^[158-161]. Furthermore, historically it was thought that terminally differentiated Th cells seldom re-differentiate to other Th subsets, however now the plasticity between Th cells is now extensively under investigation^[162].

It has been well documented that the adaptive im-

immune system plays an important role in the development and perpetuation of the inflammatory cascade in IBD. In particular, T-cells have been shown to be key players in driving intestinal inflammation. However, a number of unresolved issues exist that need to be addressed in order to develop successful and appropriate therapeutic strategies. Recent advances have clarified the importance of the innate immune system in IBD pathobiology. Furthermore, besides anti-TNF agents, molecules targeting specific T-cell derived molecules have largely failed. This is likely due to the complexities and redundancies of cytokine networks and highlights how different each individual's immune system is in the context of their own genetics. The studies of the interactions between the different components of the innate and adaptive immune system, as well as the interactions with the intestinal microbiota, and how these interactions relate in the overwhelming context of an individual's genetics are areas that will open new horizons in the knowledge of mechanisms of gut inflammation.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Hepatocyte nuclear factor 4-alpha involvement in liver and intestinal inflammatory networks

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Abstract

Hepatocyte nuclear factor 4-alpha (HNF4- α) is a nuclear receptor regulating metabolism, cell junctions, differentiation and proliferation in liver and intestinal epithelial cells. Mutations within the *HNF4A* gene are associated with human diseases such as maturity-onset diabetes of the young. Recently, *HNF4A* has also been described as a susceptibility gene for ulcerative colitis in genome-wide association studies. In addition, specific *HNF4A* genetic variants have been identified in pediatric cohorts of Crohn's disease. Results obtained from knockout mice supported that HNF4- α can protect the intestinal mucosae against inflammation. However, the exact molecular links behind HNF4- α and inflammatory bowel diseases remains elusive. In this review, we will summarize the current knowledge about the role of HNF4- α and its isoforms in inflammation. Specific nature of HNF4- α P1 and P2 classes of isoforms will be summarized. HNF4- α role as a hepatocyte mediator for cytokines relays during liver inflammation will be integrated based on documented examples of the literature. Conclusions that can be made from these earlier liver studies will serve

as a basis to extrapolate correlations and divergences applicable to intestinal inflammation. Finally, potential functional roles for HNF4- α isoforms in protecting the intestinal mucosae from chronic and pathological inflammation will be presented.

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Key words: Hepatocyte nuclear factor 4-alpha; Inflammatory bowel diseases; Colitis-associated cancer; Gastrointestinal tract; Intestinal epithelium barrier; Inflammation

Core tip: Hepatocyte nuclear factor 4-alpha (HNF4- α) is an important regulator of liver and intestinal epithelial cells function. Over the last years, HNF4- α has been associated with inflammatory bowel diseases following results obtained from knockout mice and human genome-wide association studies. However, no review has been published on the subject yet and no link with its known role in liver inflammation has been discussed. This review will gather for the first time all the current knowledge about the role of HNF4- α in gut inflammation, the potential impact of its isoforms in its role and hypotheses about the possible biological mechanisms involved.

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INTRODUCTION

Hepatocyte-nuclear-factor 4-alpha (HNF4- α) is a member of the superfamily of nuclear receptors. Since its discovery and characterisation in the early 90's, HNF4- α

has been widely associated with the transcriptional regulation of hepatocyte genes specifically implicated in lipid metabolism, glucose metabolism, differentiation and morphogenesis. However, HNF4- α expression is also detected in epithelia of the pancreas, kidneys, stomach, and intestine, for which it exerts functional roles in regulating epithelial junctions and cell proliferation. In addition to these multiple known functions in epithelium, HNF4- α has been shown to play a role in inflammation processes of the liver and recent evidences suggest the involvement of this regulator in the pathophysiology of inflammatory bowel diseases (IBD). This review will focus on the recent uncovered roles for HNF4- α during intestinal inflammation with regard on conclusions that can be made from earlier studies obtained in the liver context. Moreover, since the nature of HNF4- α isoforms expressed in the liver and the intestine differs, we will highlight the potential contribution of these isoforms in the inflammation process and, to some extent, to the inflammation-associated pathology of cancer.

HNF4- α P1 AND P2 CLASSES OF ISOFORMS

HNF4- α has been generally involved in inflammation processes occurring in liver and the gastrointestinal tract. Importantly, these organs do not express the same HNF4- α isoforms, which leads to the fundamental question of whether these isoforms are functionally redundant during inflammation. *HNF4A* is coded by a single gene located on the long arm of the chromosome 20 in human^[1]. *HNF4A* locus is transcriptionally regulated through the use of two distinct promoters that are physically separated by more than 45 kb^[2]. Isoforms produced by the activity of the closer promoter are designated P1 whereas isoforms produced by the second and more distant promoter are designated P2 (Figure 1). The P1 isoforms class regroups six distinct isoforms (α 1 to α 6) that are generated through alternative splicing of HNF4- α pre-mRNA while P2 isoforms class regroups three other isoforms (α 7 to α 9) generated through the same process^[1-6]. The functional relevance for production of such a variety of HNF4- α isoforms still remains unclear. Although these classes of isoforms share 90% of homology in their overall protein structures, an important difference is noted between their respective N-terminal domains (Figure 1). This difference causes P2 isoforms to be shorter than P1 isoforms and, more importantly, to lack the cofactor interacting domain designed as activating function (AF)-1. These structural differences suggest that P1 and P2 isoforms could harbour distinct roles by interacting differently with specific cofactors and being differently regulated in specific contexts of physiological importance. In support of this, an elegant study performed in “knock-in” mice has revealed that P1-only mice generated by exon swapping are phenotypically different when compared to P2-only mice^[7].

The expression of HNF4- α is restricted to the epithelial compartment in both the liver and intestine. However, adult hepatocytes express P1-only isoforms while intestinal epithelial cells express both P1 and P2 isoforms^[8,9]. Many studies have revealed that HNF4- α expression can vary depending on the environmental context. It has been notably demonstrated that the expression profile of P1 and P2 isoforms is modified in many cancers such as hepatocellular carcinoma where P1 isoforms expression is inhibited and P2 isoforms re-expressed^[8]. Modulation of HNF4- α isoforms expression is the result of a complex regulatory circuit that involves transcriptional, post-transcriptional and post-translational mechanisms. For instance, P1 and P2 promoters are regulated by different transcription factors^[10-12] and are differently targeted by epigenetic regulation^[10]. In addition, HNF4- α mRNA is targeted by multiple miRNA^[13-16] and its protein function and stability by phosphorylation^[17-22], acetylation^[23] and nitrosylation^[24]. Although most of these studies did not specifically investigate whether P1 or P2 isoforms share the same post-translational modifications, it is predictable that some of them could be isoforms specific. Indeed, it has been recently demonstrated that HNF4- α phosphorylation by the Src kinase preferentially targets P1 isoforms to lead to their degradation without influencing P2 isoforms stability^[25]. Thus, HNF4- α isoforms production is regulated by a wide variety of mechanisms that will connect their cellular functions to environmental specific needs.

HNF4- α ACTIVITY IS A TARGET FOR CYTOKINES EFFECTS ON HEPATOCYTES

HNF4- α is crucial for the early embryonic development and function of the adult liver as supported with the generation of mouse models with specific and conditional deletion of HNF4- α in hepatocytes^[26,27]. There is an overall agreement on the idea that HNF4- α could represent a central regulator of gene transcription in hepatocytes, making it thus a crucial transcription factor in liver physiology^[28-31]. The liver is strongly involved during the acute-phase response (APR) by the synthesis of many acute-phase proteins. Therefore, it is not surprising that HNF4- α has been confirmed to play an important role in inflammation through the regulation of acute-phase protein gene transcription. In fact, many studies demonstrated that HNF4- α is massively targeted by cytokines under these conditions. There is a general consensus supporting a negative regulatory role for cytokines on transcriptional HNF4- α action on its target genes. However, some exceptions highlight the fact that these cytokines effects could be sometimes context dependent. Studies performed in the liver hepatocellular cell line HepG2 support this phenomenon. In the context of an inflammatory redox state, interleukin (IL)-1 was shown to stimulate p38MAPK-dependent phosphorylation of HNF4- α and to increase its affinity to DNA as well as to the PC4 cofactor^[32]. This had

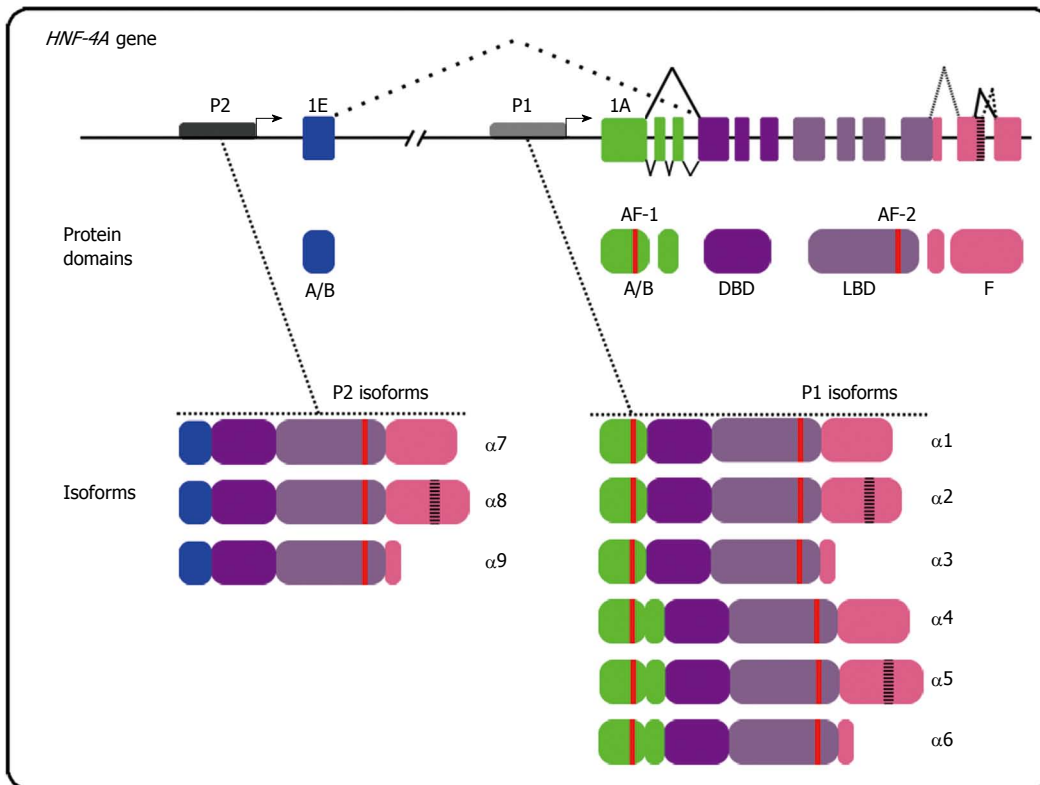


Figure 1 Hepatocyte nuclear factor 4- α P1 and P2 isoforms classes originate from alternative promoters and splicing. HNF4- α contains two distinct promoters (P1 and P2) that drive expression of nine known isoforms ($\alpha 1$ to $\alpha 9$). Transcription through the P1 promoter allows the inclusion of the exon 1A coding for the N-terminal domain of HNF4- α . P1 isoforms class displays a N-terminal region containing the cofactor interacting domain designed as AF-1. Transcription through the P2 promoter allows the inclusion of the exon 1E but the exclusion of the exon 1A. P2 isoforms class displays a smaller N-terminal domain than P1 isoforms and does not contain the AF-1 region. Alternative splicing of the last exons of HNF4A modifies the regulating F domain of both isoforms classes while alternative splicing of exon 1A modifies only A/B domain of the P1 isoforms. HNF4- α : Hepatocyte nuclear factor 4- α ; DBD: DNA binding domain; LBD: Ligand binding domain; AF-1: Activating function-1; AF-2: Activating function-2.

the consequence of increasing iNOS expression in the redox context^[32]. On the other hand, IL-1 was shown to promote the reduction of HNF4- α mRNA and protein levels in the same cell line through c-Jun effect on the P1 promoter as well as through proteasomal degradation^[33]. This effect was reported to occur only for a transient period of time and to resume to normal HNF4- α level after 12 h of treatment. Similar opposite examples apply to the pro-inflammatory role of transforming growth factor (TGF)- β , a cytokine released by the liver during stress or injury. On one hand, TGF- β is able to reduce the expression of its target genes by inhibiting HNF4- α ^[24,34]. Indeed, TGF- β stimulation in hepatic cell lines activate a signalling cascade where Extracellular signal Regulated Kinase 5 (ERK5) will phosphorylate and inactivate Glycogen Synthase Kinase (GSK)-3 β kinase that usually phosphorylates HNF4- α , a step that will promote HNF4- α interaction with target gene promoters^[35]. In addition, the TGF- β pathway causes post-translational modifications of HNF4- α that ultimately lead to its degradation by the proteasome^[24]. By opposition to these observations, HNF4- α can also potentiate TGF- β signalling pathway and its downstream effect on gene transcriptional activation. More than the third of identified SMAD2/3 binding regions in HepG2 genes

displayed an overlapping binding site for HNF4- α under specific TGF- β influence^[36]. Coincidentally, binding of HNF4- α to these SMAD2/3 dependent gene promoters was able to transcriptionally synergize them, as illustrated for the Mix paired-like homeobox (MIXL1) gene^[36].

Another argument to sustain HNF4- α involvement during hepatocyte inflammatory stimulation relates to its reported sensitivity to the NF- κ B pathway, a crucial signalling relay that dictates cellular inflammatory response. For example, it has been reported that stimulation of HepG2 cells with tumour necrosis factor (TNF)- α inhibits Apolipoprotein C3 expression through the influence of NF- κ B that targets HNF4- α DNA binding affinity and transactivation activity under these circumstances^[37]. Other evidences suggest that the control of HNF4- α activity by cytokines could be at the level of its natural interactions with cofactors that were reported necessary to promote its optimal transcriptional activity. A study performed in HepG2 cells revealed that the induction of an acute-phase response from the concomitant action of IL-1 β , TNF- α and IL-6 reduced the expression of HNF4- α -dependent APR genes by inhibiting its interaction with the coactivator Peroxisome proliferator-activated receptor-gamma coactivator-1^[38]. Although most of these studies have been performed *in cellulo*, there is also

evidence that these mechanisms are relevant in a physiological context. In support of this, Bauzá *et al.*^[39] showed that the induction of APR in mice following a burn injury was leading to an increase of IL-6 serum level and a concomitant decrease of liver HNF4- α capacity to bind to its DNA response element.

HNF4- α PROTECTS THE INTESTINAL EPITHELIUM AGAINST INFLAMMATION

HNF4- α regulates many intestinal epithelial functions. Acting as a morphogen, HNF4- α expression was shown to be required during mouse embryonic colon development^[40]. In adult, HNF4- α was also reported to be important in sustaining proper intestinal epithelial cell differentiation^[41-43], lipid metabolism^[44,45] and epithelial junctions^[29,43,46]. With such described roles, HNF4- α is now considered as an important regulator of intestinal epithelial cell homeostasis and mucosal barrier integrity. Since perturbation of the epithelial barrier is a recognized important step for initiation of IBD, HNF4- α appeared to be a logical candidate gene to be investigated in relation to these inflammatory diseases.

The first suggestion implying HNF4- α as a possible protector during IBD came from the analysis of ulcerative colitis (UC) and Crohn's disease (CD) patient biopsies where its expression was found drastically reduced as compared to non-disease controls^[47,48]. While it was not clear whether HNF4- α reduction of expression was the cause or the consequence of inflammation, it nevertheless pointed out that the expression of this transcriptional regulator could be influenced during intestinal inflammation. The generation of intestine specific HNF4- α null mouse models was able to partly resolve this question. Although it was chronologically concluded that deletion of intestinal HNF4- α did not lead to significant defects in young adult mice, these mice were found to be prone to increased susceptibility to dextran sulfate sodium (DSS) model of colitis based on the record of clinical and morphological features of the disease^[48]. The exact nature of the mechanisms involved in such susceptibility remained however speculative in this context. It was further discovered that mice lacking intestinal expression for both HNF4- α P1 and P2 isoforms were able to develop, on the long term, spontaneous inflammation similar to human IBD^[47]. The first signs of inflammation appeared around 6 mo of age and consisted of important leucocytes infiltration in the colonic mucosa. The inflammation worsened with time with the appearance of regions of acute inflammation and epithelial destruction, crypt hyperplasia and, in rare cases, early signs of neoplasia^[47]. Many cytokines were still induced in the colon of mutant mice of 12 mo of age, supporting then a chronic status of the disease. The progressive appearance of inflammation in this model suggested that the loss of HNF4- α did not immediately provoke IBD, but rather induced modifications in the epithe-

lium that eventually tilted the balance toward chronic inflammation. Exact nature of the molecular cascades involved in this progressive state of disease remains unclear. Early modification of claudin-15 expression in the mutant mice, a direct gene target of HNF4- α , suggested that an alteration in ionic transport could be part of the processes^[47]. While all these evidences suggest that long-term reduction of HNF4- α activity promote IBD, it remains unclear whether mechanisms are in place to down-modulate HNF4- α expression from the action of inflammatory signals. DSS-induced colitis caused a reduction in colonic HNF4- α expression at both the transcript and protein levels^[47,48]. As exemplified above, the influence of cytokines on HNF4- α activity is well documented in the liver but remains to be explored in the gut.

Whether these mouse studies could imply that interference on HNF4- α integrity can predispose to IBD in humans still remain an active debate. Interestingly, a human genome-wide association study has identified *HNF4A* locus as a susceptibility gene for ulcerative colitis^[49], a finding that was corroborated by an independent study using a Dutch cohort^[50]. In addition, the single-nucleotide polymorphism rs1884613 found in the P2 promoter of *HNF4A* has also been associated with the risk to develop pediatric CD^[51]. The functional relevance of these findings on a human biology perspective awaits to be addressed.

HNF4- α IS IMPLICATED IN AN INFLAMMATORY-CANCER REGULATION LOOP

HNF4- α is implicated in liver and intestine inflammation. Sustained inflammation can favour cancer development in both tissues and HNF4- α represents a strong candidate to be involved in linking these processes. An elegant study by Hatziaepostolou *et al.*^[16] supported a functional role for HNF4- α during inflammation-associated liver cancer. This study identified HNF4- α as a repressor of the inflammatory IL-6/STAT3 pathway. HNF4- α was able to maintain STAT3 in an inactive state by inhibiting the expression of the IL-6 receptor (IL6R) through activation of miR-124 transcription (Figure 2). However, when the expression of HNF4- α was inhibited in non-transformed immortalized human hepatocytes, STAT3 was activated to turn up expression of miR-64 and miR-629 that eventually turned down HNF4- α mRNA level. This kept HNF4- α expression inhibited in order to prevent its retro-inhibition feedback on the STAT3 inflammatory pathway. It was thus concluded that when this inflammatory molecular feedback loop is altered, hepatocytes initiate transformation that will contribute to cancer. Such molecular pathways linking HNF4- α to inflammation-associated cancer have not yet been identified in the intestine. However, a recent study from Koukos *et al.*^[52] reported a similar mechanism where STAT3

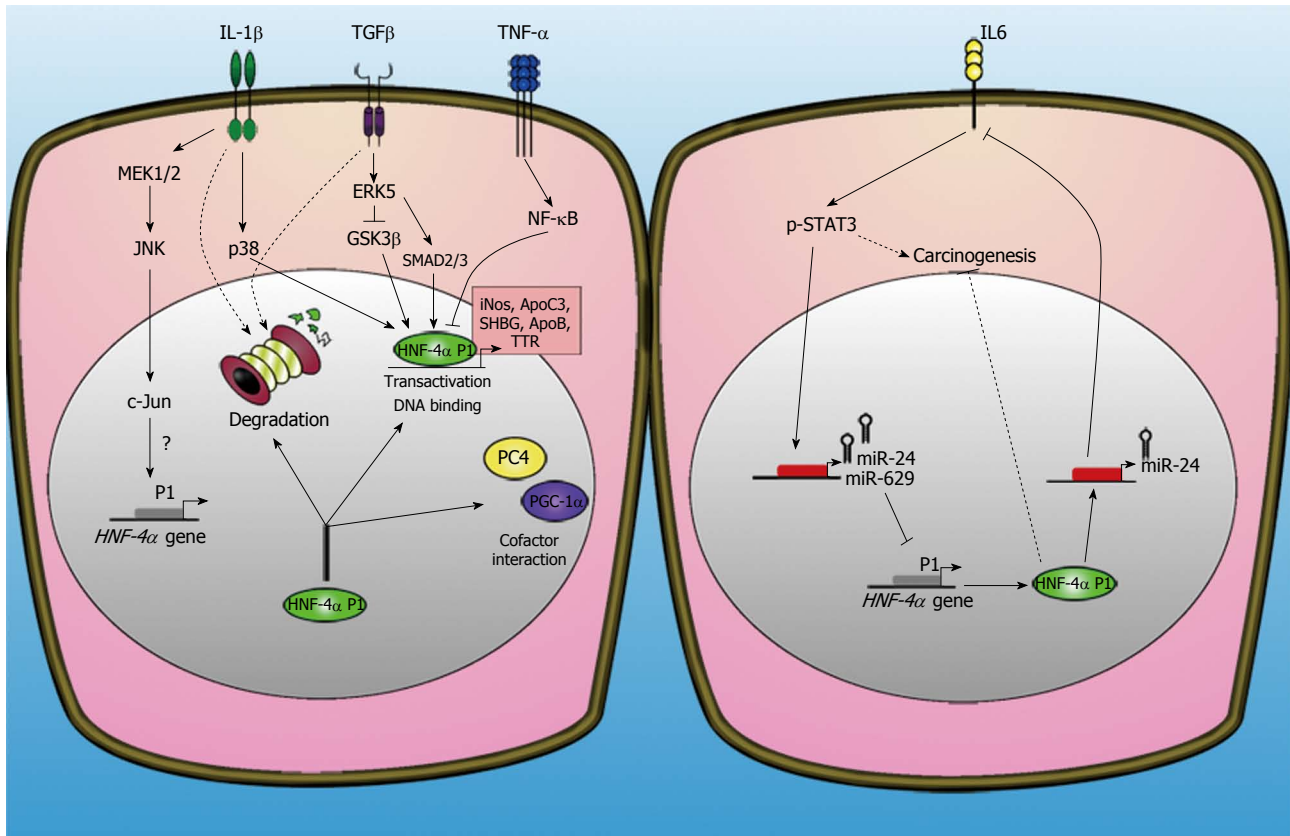


Figure 2 Cytokines effect on hepatocyte nuclear factor 4-alpha activity and inflammation associated-cancer in hepatocytes. Cytokines regulate hepatocyte nuclear factor 4-alpha (HNF4- α) function through its proteosomal degradation, DNA binding affinity, transcriptional activity and cofactor interaction (left panel). HNF4- α participates also to a retro-inhibition feedback loop of the oncogenic interleukin (IL)-6/STAT3 pathway (right panel). In hepatocytes, HNF4- α binds to miR-124 promoter and activates its transcription. MiR-124 targets IL-6 receptor transcript to decrease its expression and then block the activation of IL-6 pathway. On the other hand, stimulation of hepatocytes by IL-6 activates the phosphorylation of STAT3 that binds and transactivates the promoters of miR-24 and miR-629. These two microRNA target HNF4- α transcript and decrease its expression to relieve inhibition of the IL-6 pathway. The perturbation of this molecular circuit ultimately results in the sustained activation of the oncogenic STAT3 pathway and the loss of the tumour suppressor effect of HNF4- α P1 isoforms.

activity was also under the control of miR-124 in the context of pediatric UC. It is thus logical to extrapolate that HNF4- α could also inhibit the IL6/STAT3 pathway through regulation of miR-124 expression in intestinal epithelial cells. Indeed, DSS-induced colitis resulted in a decrease of HNF4- α expression in intestinal epithelial cells^[47,48] while it increased STAT3 mRNA level^[52]. By extension, colorectal cancer is associated with a decrease of HNF4- α P1 isoforms expression^[8,25] and activation of the STAT3 pathway through IL-6^[53,54]. The existence of such a retro-inhibition loop of HNF4- α on the IL6/STAT3 pathway represents thus an interesting possibility to explore in the context of IBD and colitis-associated colorectal cancer.

The results obtained from Hatziaepostolou *et al.*^[16] suggest that the inflammatory-cancer regulation loop could be dependent on HNF4- α P1 isoforms. While the authors did not specifically establish which isoforms were expressed in their models, it is well documented that P1 isoforms are the major ones expressed in hepatocytes as well as hepatocyte-derived cultured cells. Whether P2 isoforms could also be involved in the inflammatory-cancer loop of IL6R/STAT3/HNF4- α will be important to establish. In hepatocellular carcinoma, the

expression of P1 isoforms is reduced while it has been reported that P2 isoforms are often transiently up-regulated^[8,55]. In addition, the intestinal epithelium expresses both P1 and P2 isoforms. In that context, it is tempting to extrapolate that P1 isoforms would exert tumour suppressor roles in hepatocytes^[56-58] and intestinal epithelial cells while P2 isoforms would assume different roles.

CONCLUSION

HNF4- α appears to be a transcriptional sensor of inflammation and some of the mechanisms and interacting pathways involved in the liver context have been elucidated. Although there are growing evidences for intestinal epithelial HNF4- α to participate in the pathophysiological consequences of gut inflammation, the exact molecular and biological mechanisms involved are still to be defined. There are several non-exclusive hypotheses to explore the functional roles for HNF4- α in this context (Figure 3). As observed in hepatocytes, intestinal epithelial HNF4- α could sense and regulate cytokines effect on gene transcription. In addition, HNF4- α could play an active role in the maintenance of adequate mucosal barrier properties. HNF4- α could

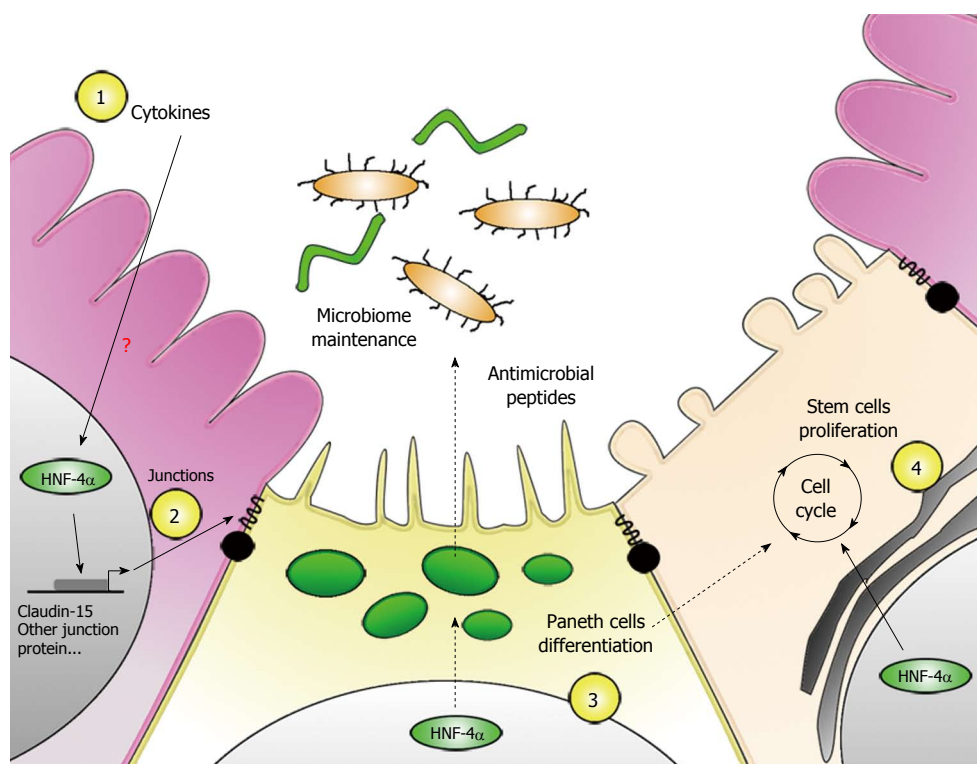


Figure 3 Hepatocyte nuclear factor 4- α potential physiological roles in protecting the gut against inflammation. There are several hypotheses for the functional roles of hepatocyte nuclear factor 4- α (HNF4- α) in the context of intestinal inflammation. As observed in hepatocytes, intestinal epithelial HNF4- α could sense and regulate cytokines effect on gene transcription (1). Among others, HNF4- α could influence mucosal barrier properties by regulating the expression of cell junction proteins such as claudin-15 (2). Moreover, HNF4- α could influence microbiota homeostasis through its role on Paneth cells differentiation (3) and goblet cells mucins expression (not shown). Finally, HNF4- α could also be implicated in the maintenance of the regenerative properties of the mucosal barrier. It could participate to stem cells proliferation through maintaining Paneth cells differentiation and through indirect regulation of the β -catenin pathway (4).

also indirectly control the microbiota to prevent the progressive emergence of chronic inflammation based on previous results showing that Paneth cells integrity is affected in HNF4- α knockout mice^[41]. Finally, HNF4- α could be part of regulatory signals closely involved in the maintenance of the regenerative mucosal properties during chronic injuries (Figure 3). In contrast to hepatocytes that express P1-only isoforms, intestinal epithelial cells express both P1 and P2 isoforms. It is plausible that these isoforms harbour distinct roles during intestinal inflammation based on the fact that inflammation can influence HNF4- α activity through cofactors interaction^[38] and that P2 isoforms lack the cofactor interacting domain AF-1. Moreover, the fact that HNF4- α isoforms are differently expressed in the course of intestinal diseases such as colorectal cancer^[8,25] strengthens the idea that this might occur during inflammation. Exploration of these possibilities will turn out to be of clinical relevance in the context of targeting HNF4- α for diagnostic and/or therapeutically strategies designed to control pathophysiological conditions of IBD.

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Natural history and long-term clinical course of Crohn's disease

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Abstract

Crohn's disease is a chronic inflammatory disease process involving different sites in the gastrointestinal tract. Occasionally, so-called metastatic disease occurs in extra-intestinal sites. Granulomatous inflammation may be detected in endoscopic biopsies or resected tissues. Genetic, epigenetic and environmental factors appear to play a role. Multiple susceptibility genes have been described in both familial and non-familial forms while the disease is phenotypically heterogeneous with a female predominance. The disorder occurs over a broad age spectrum, from early childhood to late adulthood. More than 80% are diagnosed before age 40 years usually with terminal ileal and colonic involvement. Pediatric-onset disease is more severe and more extensive, usually with a higher chance of upper gastrointestinal tract disease, compared to adult-onset disease. Long-term studies have shown that the disorder may evolve with time into more complex disease with stricture formation and penetrating disease complications (*i.e.*, fistula, abscess). Although prolonged remission may occur, discrete periods of symptomatic disease may re-appear over many decades suggesting recurrence or re-activation of this inflammatory process. Eventual development of a cure will likely depend on identification of an etiologic cause and a fundamen-

tal understanding of its pathogenesis. Until now, treatment has focused on removing risk factors, particularly cigarette smoking, and improving symptoms. In clinical trials, clinical remission is largely defined as improved numerical and endoscopic indices for "mucosal healing". "Deep remission" is a conceptual, more "extended" goal that may or may not alter the long-term natural history of the disease in selected patients, albeit at a significant risk for treatment complications, including serious and unusual opportunistic infections.

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Key words: Natural history; Crohn's disease; Age-dependent phenotypes

Core tip: Crohn's disease remains an intriguing heterogeneous disorder characterized by a granulomatous inflammatory process. The phenotypic expression of Crohn's disease is clearly age-onset dependent as most children and adolescents suffer more severe, more extensive and more complicated disease than most adults, and the elderly. If evaluated over a long period of time, the disease appears to be progressive, but only intermittently active, with some appearing to have prolonged periods of sub-clinical disease and others expressing complex disease with stricture formation and penetrating complications, even at the time of initial clinical presentation. Although the precise cause of Crohn's disease remains a mystery, an increasing appreciation for the long-term natural history may permit development of more effective treatment regimens. Ultimately, however, both clinical and fundamental investigative efforts should focus on discovering the cause of the disorder since this approach may offer the best opportunity for cure.

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INTRODUCTION

Crohn's disease is a chronic inflammatory disorder that usually involves different sites along the length of the gastrointestinal tract, and also, occasionally, other extra-intestinal "metastatic" sites. Genetic, epigenetic and environmental factors are believed to play a role in its etiology and pathogenesis^[1,2]. Indeed, recent evidence suggests that multiple susceptibility genes may be present and play a critical role^[1,2]. Some believe that genetic risk profiling to predict the eventual disease course is feasible^[3,4]. Currently, it is thought that the disorder is likely related to an aberrant immunological response to intestinal microbiota in genetically-susceptible individuals^[5]. A prevalence of up to 1% has been recorded with numerous studies suggesting that the incidence is increasing in developed or industrialized as well as developing or non-industrialized countries^[6]. Although there is little epidemiologic data, a recent analysis noted that the rise in incidence and prevalence over time and in different regions around the world reflects emergence as a global disease^[6]. A key pathological footprint of Crohn's disease is the granulomatous inflammatory response, a special form of chronic inflammation characterized by focal collections of macrophages, epithelioid cells and multinucleated giant cells. It is believed that initiation and development of this specific form of inflammatory reaction depends on persistence of the inciting agent, a complex immune reaction to perpetuate the granulomatous response leading to development of necrosis and fibrosis^[7].

The clinical expression of Crohn's disease is known to be very heterogeneous. Prior working groups for the World Congress of Gastroenterology in Vienna and later in Montreal developed a classification scheme that has evolved^[8,9]. An important goal was to enumerate different phenotypic characteristics so that more homogeneous cohorts could be explored and compared, particularly for geographically-distinct populations. Application of this classification schema to a tertiary care clinical database revealed a female predominance, occasional familial nature, and a high rate of stricture formation and penetrating disease complications^[10,11].

Crohn's disease appears to be a life-long disorder becoming clinically apparent at almost any time from early childhood to late adulthood^[10,11]. For most, the actual onset of the disease, or more precisely, age of detection or diagnosis, has usually been during the late teens and early twenties, and now, during the past two or three decades, over 80% of patients with Crohn's disease are diagnosed before age 40 years^[10]. Moreover, the majority have involvement primarily of the ileum and colon, at least based on the most sensitive and most modern imaging methods^[10,11]. Finally, most clinicians usually evaluate and treat "late" complicated disease, while "early" disease

without stricture or fistula development may not be as commonly seen, particularly in tertiary-care centers.

ONSET OF DISEASE AND DIAGNOSIS

Clearly, an appreciation for the natural history of Crohn's disease must depend, not on the age at diagnosis defined by these classifications, but the actual time of onset of the disorder. These are obviously very different. Indeed, some authorities have opined that symptoms may be chronically present over a long latent period in many patients for months, even years, before a diagnosis is entertained and established. Symptoms that eventually lead the patient to specialist referral include diarrhea, abdominal pain and weight loss, but these are not always universally present. Occasionally, only a single symptom, possibly abdominal pain alone, may be evident. In some, suspected appendicitis may first lead to detection of an unexpected ileal inflammatory process. For others, extra-intestinal findings (*e.g.*, arthropathy or a skin disorder, such as erythema nodosum) may be present without significant abdominal symptoms. Or, rarely, granulomatous inflammatory change defined in an extra-intestinal site, called metastatic Crohn's disease, including skin, muscle, or bone may complicate or actually lead to recognition of occult intestinal disease^[12-14]. For metastatic disease, a distinct focus of granulomatous inflammatory disease is defined, geographically separated from the gastrointestinal tract. Other "systemic" or distant sites may also be involved in patients with Crohn's disease leading to atypical clinical features, such as a concomitant sellar inflammatory mass^[15], or even unusual complications, sometimes attributed to treatment, such as osteonecrosis^[14].

In Crohn's disease, the initiating event (including, a possible infectious agent) leading to this ongoing, step-wise and destructive inflammatory process may no longer be detectable, having long departed the scene. Indeed, multiple events (or agents) could conceivably precipitate or generate this insidious inflammatory cascade causing eventual symptoms and signs at the bedside as well as end-pathological changes in the laboratory, clinically recognized as Crohn's disease. Additional studies are urgently needed to further elucidate these hypothetical genetic, microbiologic and immunologic factors that permit progression of this frustrating clinical disorder.

DISEASE EXTENT AND LOCALIZATION

In Crohn's disease, there is a predilection for the distal small intestine and proximal colon^[8-11]. Of course, more extensive involvement of the intestinal tract may also occur^[16] and the old adage that Crohn's disease can potentially involve any site "from mouth to anus" still holds true, although this probably deserves to be altered slightly to "and in other sites separated from the gastrointestinal tract as metastatic Crohn's disease". Familial factors play a role in Crohn's disease^[17], including genetic factors that appear to directly influence the localization

of the disease in different intestinal sites^[18,19]. Further information, however, is clearly required on fundamental luminal and intestinal factors that play a role in localization of the disorder to specific sites along the length of the gastrointestinal tract.

Crohn's disease may develop in the upper gastrointestinal tract, often with concomitant disease in the ileum, colon, or elsewhere. Rarely, at least in Caucasian populations, the disorder may also occur solely in the upper gastrointestinal tract without disease involvement elsewhere^[9-11]. Interestingly, a recent study from Hong Kong suggested that the Chinese may have an increased risk for this upper gastrointestinal tract phenotype^[20]. Also, extensive jejuno-ileal disease, evaluated over the long-term, appears to reflect a form of Crohn's disease that historically responds poorly to medications, often leading to surgical treatment, long-term nutritional support and greater costs for care^[13,20,21]. New and developing biological treatment paradigms largely focused on reducing numerical activity indices in ileocolonic disease may have little impact here, unless the long-term severity and extent of the inflammatory process can be reduced. In recent years, improved and novel imaging methods have also opened the door to more precise recognition of this extensive small intestinal group^[22], so that early inflammatory changes may be detected along the length of the small intestine, rather than late-stage and more complex disease.

DISEASE BEHAVIOR

Crohn's disease is a chronic, persistent and destructive disorder with different forms of clinical behavior^[23,24]. Over the long term, the disease appears to be progressive although the rate of progression may be altered or slowed, by the use of some medications, or with surgical treatment, at least for a period of time. Oral corticosteroids within 3 mo of diagnosis and early thiopurine use within 1 year were recently shown over several decades to independently affect the likelihood of intestinal surgery^[25]. Another long-term study suggested that immunomodulator use for over 6 mo reduced the risk for first surgery, particularly in non-stricturing non-penetrating Crohn's disease^[26]. Other long-term studies have independently suggested that the disease may start as an inflammatory process with progressive development over time to more complex disease with stricture and fistula formation^[27-29]. Once initiated, it is likely that numerous genetic and environmental factors play a role in regulating the rate of progression, but these are poorly understood. Moreover, the "progression" of the disease itself may not necessarily be a linear process but rather progression may occur in a step-wise fashion with prolonged symptom-free periods over many decades^[30].

Crohn's disease may also initially present as an already advanced and clinically complex disease with extensive or multiple jejunoileal strictures^[16], sometimes even with free perforation of the small intestine, large intra-abdominal

inflammatory masses, and deeply penetrating fistulae (*e.g.*, ileosigmoid fistula). In some, it has been hypothesized that recurrent disease may occur as a patterned clinical response, possibly related to specific genetic regulatory factors. For instance, recurrent stenotic events may result in a localized ileocecal resection, "new" erosions and ulcers in the "neo-terminal" ileum, and further stricture formation, recurrent obstructive symptoms and another resection^[31]. Or, in some, recurrent penetrating events with fistula and abscess formation may occur^[31].

Recently, additional long-term studies of post-operative Crohn's disease have been reported^[32-34], even here, cigarette smoking has been again identified as the strongest risk factor for recurrence^[34]. Clinical experience has also shown that classifying clinical behavior in Crohn's disease is difficult and may not be truly reflective of natural history as the rates of development of a complication, such as a stricture, may differ remarkably, not only between different patients, but even in the same patient. Some may have either a rapidly progressive inflammatory process, or alternatively, a low grade sub-clinical process, possibly present for months, that suddenly becomes clinically expressed. A recent report noted that 10 years following a resection for ileo-cecal disease, only about half were free of clinical recurrence and about 30% needed added surgical treatment^[32].

AGE-RELATED PHENOTYPIC EXPRESSION

Early historical studies suggested that the phenotypic clinical expression of Crohn's disease differed substantially, depending on the age of initial diagnosis^[35-44]. This age-dependent phenotypic clinical expression probably, in part, reflects an age-dependent regulation of the inflammatory process^[40]. Disease developing earlier in children and adolescents tends to be much more severe, often resulting in significant disease complications, including strictures or fistulae, or both^[40-45]. It is also more extensive in childhood, often involving multiple sites in the small and large intestine, with a higher frequency of involvement of the upper gastrointestinal tract^[40-45]. Comparative studies also show significant differences in clinical expression between children and adults^[41,42] as well as the elderly^[46,47]. Others have defined a difference in some "immune-reactive" characteristics of early-onset compared to late-onset Crohn's disease^[48-50]. A hypothesis that suggests that a dysregulated immune response occurs, likely affected by aging *per se*, and leading to different phenotypic disease expressions of Crohn's disease, needs to be further elucidated.

CLINICOPATHOLOGICAL CORRELATIONS

Clinical and pathological correlations have been explored during the long-term clinical course of Crohn's disease. Some early historical descriptions tended to avoid the pos-

sible temporal sequence of progression of pathological lesions and focused on their prognostic significance^[51,52]. However, more recent studies related to this chronological sequence have hypothesized that early small ulcers or granulomas initially develop with ongoing progression to later sinuses and strictures complicating large ulcers^[53]. These have also confirmed that this granulomatous inflammation may be a histopathological marker for an early phase of the inflammatory process in Crohn's disease, at least prior to development of fibrotic strictures and fistulous tracts^[53]. Granulomatous inflammatory change has been documented in multiple endoscopic biopsies and surgically-resected specimens obtained over many decades from the same patient^[29,54]. Often, long intervals of relatively symptom-free disease may be documented^[29,54]. This temporal pattern may indicate multiple initiating events in Crohn's disease with different rates of progression, or alternatively, the presence of granulomas may simply reflect ongoing active inflammation, even with subclinical or asymptomatic disease.

These studies also suggested a geographic change in the location of detectable Crohn's disease along the length of the gastrointestinal tract with extended periods of time^[29]. For instance, granulomatous ileal involvement was defined many years after initial colonic disease, and gastroduodenal disease occurred after ileocolic resection. If there was a single event that initiated this granulomatous inflammatory process, then different tissues along the length of the gastrointestinal tract may develop a granulomatous response at different rates, or alternatively, there might be multiple or recurring initiating events. Possibly, different gastrointestinal sites may differ in sensitivity to a possible initiating event (or infectious agent). This could be due to a site-specific differential in intestinal permeability or differing immunological responses along the length of the gastrointestinal tract^[55,56].

SUMMARY AND FUTURE CONSIDERATIONS

Changes in the clinical features, course and prognosis of inflammatory bowel disease during the last 5 decades has been recently evaluated^[57]. In spite of variations in presentation and initial course, the long-term prognosis for Crohn's disease appeared to be stable. A database review for a similar time period noted that about a third of patients had ileitis, colitis or ileocolitis at the time of diagnosis. After about 20 years, half suffered an intestinal complication. Only 10% had a prolonged clinical remission. About a third required steroid therapy to some extent and a third needed surgical treatment after steroids were used. Hospitalization was necessary in 20% and about half needed surgery within 10 years from diagnosis^[58]. Further studies to examine the effects of different therapies on the natural history of Crohn's disease are in early stages. A long term evaluation of a population-based inception cohort from Hungary documented a reduction in surgical rates associated with increased and

earlier use of azathioprine^[59]. Although some consider endoscopic mucosal healing as a useful target in ulcerative colitis, the role on the long-term natural history of Crohn's disease has been more difficult to establish and is not known. Additional information on the effects of biological agents, especially TNF blockers^[60], on the long-term natural history is critical. There is evidence that these agents may reduce disease complications requiring surgical treatment, but a significant adverse risk profile remains, particularly for serious opportunistic infections^[61]. Some have hypothesized that directing focus to induction of a "deep remission" may have potential to change natural history^[62], even if the precise cause of Crohn's disease is not defined. Time will tell.

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Gastrointestinal motility disorders in inflammatory bowel diseases

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Abstract

The relationship between motility and inflammatory gastrointestinal disorders is at the same time complex and intriguing since these conditions might share some genetic, environmental, immunological and microbial predisposing factors. In addition, significant symptom overlapping may occur, muddling the waters within the clinical context. Although on one hand this represents a challenge for the clinician for a potential under- or over-treatment and diagnostic delay, on the other hand it possibly represents an opportunity for the researcher to better disclose the intimate relationship between chronic (often low-grade) inflammation, motor disorders and deranged sensory function. The best example is probably represented by Crohn's disease and ulcerative colitis. In fact, a number of gastrointestinal motor disorders have been described in association with these diseases, disorders which span

from the esophagus to the anorectum, and which will be extensively covered in this review. It is conceivable that at least part of this derangement is strictly related to inflammatory cytokine trafficking and neuromuscular changes; however, given the high prevalence of functional gastrointestinal disorders in the general population, this overlap might also be serendipitous. However, it is worth noting that literature data on this topic are relatively scarce, sometimes quite outdated, and mostly focused on the interplay between irritable bowel syndrome and inflammatory bowel disease. Nevertheless, both researchers and clinicians must be aware that symptoms related to gastrointestinal motility disorders may be highly prevalent in both active and inactive inflammatory bowel disease, correlate with greater psychological comorbidity and poorer quality of life, and may negatively influence the therapeutic approaches.

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Key words: Crohn's disease; Gastrointestinal motility disorders; Gut; Inflammatory bowel diseases; Perception; Ulcerative colitis

Core tip: Gastrointestinal motor disorders are not infrequently associated with inflammatory bowel disease and may represent a confounding factor, especially when inflammation has subsided or the clinical picture is in remission. Since these entities may involve all the segments of the gastrointestinal tract, it is important that clinicians and researchers be aware of this potential overlap, since lack of knowledge may lead to mistreatment or overtreatment. However, literature data on this topic are relatively scarce and are extensively covered in this review.

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INTRODUCTION

Inflammatory bowel diseases (IBD), mainly represented by Crohn's disease (CD) and ulcerative colitis (UC), are emerging pathological conditions whose epidemiological rising is thought to be related to the westernization of lifestyle and industrialization^[1]. The genesis of IBD is quite complex and numerous pathophysiological factors are now recognized to be involved in the predisposition, triggering, progression and outcome of these conditions^[2].

Interestingly, several of these factors are the same thought to be responsible for the genesis of (at least some of) gastrointestinal motor abnormalities, disorders that are classified among the so-called functional gastrointestinal disorders^[3], *i.e.*, those pathological entities in which no known structural (pathological or radiological) abnormalities, infectious or metabolic causes are present^[4]. However, in recent years it has become evident that, at least for some of these entities, the pathophysiological mechanisms, defined as "idiopathic" until now, can actually be reconducted to some form (sometimes very subtle) of low grade inflammation. This is, for instance, the case of esophageal achalasia^[5,6], functional dyspepsia^[7,8], irritable bowel syndrome (IBS)^[9] and chronic constipation^[10,11], where the presence of limited number of inflammatory cells infiltrating the mucosa and/or the myenteric plexus has been reported. On the other hand, the recent description of enteric nervous system (ENS) abnormalities in IBD patients (mainly represented by myenteric plexitis)^[12,13] may justify alterations of the normal motor function and visceral reflexes of the gut.

Thus, as we will see below, the co-existence of IBD and gastrointestinal motor disorders may somewhat represent more than a serendipitous occasion and it is likely that motility and/or perception abnormalities play a role in the clinical pictures of IBD, sometimes complicating the physician's assessment and the patient's lifestyle. To what extent these motor abnormalities provoke or correlate with gastrointestinal symptoms reported by IBD patients during remission is rather unknown. However, despite potential limitations, the majority of studies report a greater frequency of "IBS-like" symptoms in quiescent IBD compared to the background population^[14].

GENERAL CONSIDERATIONS

The relationship between gastrointestinal motility disorders and IBD is a quite complex one and not well elucidated yet since multiple variables appear to play a role. For instance, IBS-like symptoms are frequently found in patients with IBD considered to be in clinical remission and it is very difficult to distinguish these symptoms as due to IBS or to persisting occult inflammation^[15]. More-

over, symptoms related to motility/functional gastrointestinal disorders are highly prevalent in both active and inactive IBD and correlate with greater psychological comorbidity and poorer quality of life^[16,17]. Some data also suggest that intercurrent infectious gastroenteritis may increase IBD risk in IBS patients^[18]. However, as can be seen from the literature evidence, most data on the relationship of functional gastrointestinal disorders/IBD is actually focused on the relationship of IBS/IBD and the other gastrointestinal motor abnormalities are relatively neglected. The following paragraphs will take into consideration these relationships for each segment of the gastrointestinal tract (Table 1).

ESOPHAGUS

Although relatively uncommon (about 5%-8% in both adults and children^[19,20]), esophageal involvement has become increasingly recognized in IBD, especially in patients with CD^[21], even in the absence of specific symptoms as patients more frequently undergo upper endoscopic evaluations^[22]. The clinical manifestations are usually due to the mucosal and/or transmural involvement by the inflammatory process. One report showed an achalasia-like motility disorder in a patient with dysphagia and CD, with the esophageal dysmotility being due to the extensive inflammatory/fibrotic process^[23]. To date, no specific clinical or manometric investigations have been carried out in IBD patients, except a small group of UC patients undergoing esophageal manometry as a pathological control group^[24] in whom no abnormalities were found compared with normal controls.

It is worth noting that gastro-esophageal reflux symptoms are more frequent in patients with active IBD compared with those with quiescent disease^[25] and are associated with a reduced quality of life and increasing likelihood of anxiety and depression compared to controls^[26].

STOMACH

Upper gastrointestinal involvement in IBD patients may be found in about 15%-20% of patients^[27,28]. However, subtle microscopical abnormalities of the stomach and duodenum may be also present in patients with IBD and might explain some upper gastrointestinal symptoms^[29]. However, to date, only a few studies have investigated gastric motor function in patients with IBD. An earlier study with barium meal (a suboptimal method to evaluate gastric motor activity) on three patients with gastric involvement showed loss of normal gastric motility^[30]. A subsequent scintigraphic study on pediatric patients showed that gastric emptying was normal in UC patients and delayed in about 30% of children with CD^[31]. This delay was more prominent in malnourished children^[32]. The scintigraphic findings of normal gastric emptying in UC adults have been confirmed by other authors^[33]. Gastric emptying after intestinal resection in adults with CD proved to be within normal limits and the authors

Table 1 Summary of motor abnormalities and gastrointestinal symptoms in inflammatory bowel diseases patients in each segment of the gastrointestinal tract

Segment	Motor changes	Symptoms	Ref.
Esophagus	Aspecific, achalasia-like (rare)	Gastroesophageal reflux	[18,19,24,25]
Stomach	Delayed emptying	Not reported (?)	[30,31,34,35]
Biliary tree	Possible reduction of cyclic contractions of gallbladder due to deranged phase III and motilin peaks	Symptoms related to gallstones	[41,42]
Small bowel	Large amplitude, rapidly propagated waves in small bowel of UC after proctocolectomy	IBS-like symptoms	[49,57-60]
	Reduced small bowel contractions, increased incidence single and clustered propagated contractions in CD		[63,64]
Colon	Decreased contractility in UC with accelerated transit.	Diarrhea	[75-77]
	Reduced tone after meals		[80]
	Increased propulsive activity		[81]
Anorectum	Low anal pressure, poor rectal distensibility, reduced compliance, enhanced perception	Incontinence, urgency	[84-88] [90-93] [94,95]

UC: Ulcerative colitis; CD: Crohn's disease.

concluded that the diarrhea observed in these subjects should not be attributed to rapid gastric emptying^[34]. In a scintigraphic study on gastric emptying in adult patients with non-obstructive CD and no upper gastrointestinal involvement, we showed that, compared with healthy volunteers, no overall significant differences were found; however, subgroup analysis revealed that symptomatic patients and those with colonic involvement had a significantly delayed gastric emptying compared to controls. Such a difference was also observed between symptomatic and asymptomatic patients^[35]. Another scintigraphic study investigated the occurrence and putative pathophysiological mechanisms of gastric motor disorders in a small group of adult IBD patients and concluded that disturbances of gastric emptying are most pronounced in CD and might partly be caused by excessive CCK release^[36].

BILIARY TREE

Although the hepatobiliary tree is frequently affected in patients with IBD^[37], its motor function has been scarcely investigated in these subjects and only a few data are available in the literature. An ultrasound study carried out in a small group of IBD patients during continuous enteral nutrition revealed that gallbladder contractility and responsiveness to intravenous cholecystokinin are preserved in such instances^[38]. Another ultrasound study reported that after ileo-cecal resection, fasting gallbladder volume is decreased, whereas postprandial gallbladder motility is normal^[39]. Cholecystectomy specimens from patients with IBD did not show significant histopathological differences compared to controls^[40]. Nevertheless, gallstone prevalence is increased in CD patients; site and duration of disease and previous intestinal resections are considered as risk factors, even although data are conflicting^[41]. In 25 asymptomatic, uncomplicated CD patients we found a significant derangement of phase III of migrating motor complex in terms of occurrence, cycling and origination from the antro-duodenal region. Moreover, no clear peaking of motilin before phase III

was found^[42]. Therefore, we hypothesized that in some patients with CD, a reduction of cyclic phasic contractions of gallbladder occurs because of a decreased incidence of motilin peaks and phases III of the migrating motor complex in the antro-duodenal area. Thus, the lack of periodic stirring of bile contained in the gallbladder may lead to supersaturation and stone formation^[43].

SMALL BOWEL

The small bowel represents the main anatomic site of involvement for patients with CD, even although the terminal ileum may be rarely involved in UC^[44]. *In vitro* studies showed that inflammation impairs the contractile responses of human small intestine^[45] and this may be observed even with minimal inflammatory responses^[46].

Thus, IBD patients may complain of symptoms not only related to the active phases of their disease, but also to low grade inflammation that produces symptoms correlated to autonomic dysfunction and abnormalities of the motor activity or of visceral sensations^[47]. These symptoms are associated with lower quality of life and higher healthcare utilization in IBD patients and may herald a cohort at risk for worse outcomes^[48] since they may be present even in patients with quiescent disease, in whom the quality of life may be severely impaired by this association^[49,50]. In addition, in these patients a potential overtreatment with drug-related toxicity might occur. On the above grounds, it is therefore not surprising that a substantial number of IBD patients experience IBS-type symptoms (abdominal pain and discomfort, diarrhea, constipation)^[14], symptoms that often originate from low-grade inflammation causing abnormal motility and perception, especially (but not only) in the small intestine^[51]. These patients exhibit similar pathophysiological features to people diagnosed with IBS in the general community^[52-54], suggesting that the conditions are not mutually exclusive, may coexist in a considerable number of IBD patients^[55], and that clinical indexes may be insufficient to discriminate between the two conditions^[56].

Fortunately, the availability of fecal markers of inflammation may be quite useful in this setting^[57]. However, it should be kept in mind that a certain number of IBD patients with normal fecal calprotectin levels experience IBS-type symptoms and exhibit similar features to people diagnosed with IBS in the general community, suggesting that the conditions are not mutually exclusive and may co-exist in a considerable number of IBD patients^[55].

Concerning objective measurements, it is worth noting that, notwithstanding the frequent involvement, the small bowel motor function (due to the relatively invasive manometric techniques needing deep intestinal intubation and prolonged periods of recordings) has been scarcely investigated in IBD.

UC

The few available studies have been conducted on the small bowel of patients after proctocolectomy and ileal pouch-anal anastomosis. In a manometric study on eight such patients, the authors concluded that jejunoileal motility is not greatly altered by this kind of surgery. However, the appearance of large amplitude, rapidly propagating waves in the proximal jejunum after operation may be a response to increased storage within and distention of the distal bowel^[58]. Subsequently, similar findings (also by electromyographic assessment) have been reported by other authors^[59,61]. Interestingly, the defunctionalized ileum in these patients is able to regain at least some of its motor function after a brief period of meal stimulation^[62].

CD

In a study conducted on nine patients with partial mechanical obstruction due to CD, jejunal manometry showed that after the meal the most striking finding was the regular occurrence of clustered contractions. The associated periods of quiescent motor activity may account for the unexpectedly reduced postprandial frequency and motility index in obstructed patients compared with normal subjects^[63]. Another manometric study, carried out in CD patients without any sign of occlusion receiving total parenteral nutrition for acute exacerbation of the disease, showed that after correction of nutritional status and clinical improvement, jejunal motility is almost normal in most patients, although some abnormalities (mainly affecting the interdigestive motor complexes) may be documented in a few subjects^[64]. In a manometric study on fasting and postprandial gastroduodenal motor activity carried out in 35 patients with inactive CD^[65], we observed abnormal motility aspects in 74% of these patients and concluded that most patients with inactive, uncomplicated CD display marked gastrointestinal motor disorders, characterized either by reduced incidence of small bowel contractions and increased incidence of single or clustered propagated contractions.

LARGE BOWEL

Since this viscus is the most frequently affected target in UC, it is not surprising that (also due to its relatively easier

access), concerning motility and perception, the researchers' interest has focused more on the colon than on the other hollow viscera. Indeed, there is wide experimental evidence that inflammation may affect the motor^[66-69] and perceptive^[70-72] function of the large bowel. Interestingly, these effects are observed after resolution of the inflammatory phenomena^[73] and might be related to the fact that, at least for a subset of patients, a complete mucosal healing is not (or never) reached^[74] and a persistent sub-clinical inflammation associated with increased colonic paracellular permeability may be associated with the presence of IBS-like symptoms^[75]. It is also worth noting that the role of inflammation is not limited to the mucosa and muscular components of the bowel wall, but also involves the neuroenteric circuitries (*i.e.*, the ENS)^[12,13,76].

Early manometric studies carried out in the rectosigmoid area revealed decreased colonic contractility in patients with UC^[77,78] and correlated this decrease with the presence of diarrhea^[79], findings also confirmed by myoelectric techniques^[80]. Subsequently, the simultaneous assessment of motility and transit in UC patients carried out by a combined manometric/scintigraphic approach confirmed that the reduced colonic motor activity is also present in more proximal segments and that these patients have increased propagated activity of the colon that may speed the transit of contents^[81]. Moreover, it has been demonstrated by studies conducted with the barostat that UC patients display reduced tone of the descending colon following meals^[82]. More recent studies evaluating 24 h manometric recordings of colonic motility in patients with UC showed that, compared to controls, patients with moderately active disease have increased propulsive activity (of both high and low amplitude), a mechanism likely to be responsible for the diarrhea^[83]. This mechanism may be related to the distension of the large bowel^[84] due to accumulation of fluids and inflammatory materials. Interestingly, the increased number of high-amplitude propagated contractions reduces in UC patients in remission, whereas the low-amplitude propulsive activity remains higher compared to controls, possibly contributing to the persistence of abdominal symptoms in a subgroup of patients^[85].

ANORECTUM

CD

Anorectal manometric variables and rectal sensation have been reported to be abnormal in CD patients, even in those with only microscopic involvement^[86-89]. In particular, low pressures due to anal involvement are strongly correlated with fecal incontinence^[90]. Other authors, however, were unable to correlate urgency with anorectal variables or sensation^[91]. It is worth noting that poor rectal distensibility may predict a worse outcome of ileorectal anastomosis^[92].

UC

The frequency and urgency of defecation and the fecal incontinence in these patients may be due to a hyper-

sensitive, hyperactive and poorly compliant rectum^[93-95]. In patients with active disease, no abnormalities of the sphincter functions are present, whereas perception to stimuli appears altered compared to controls^[96,97].

Postoperative conditions

Anorectal manometry can predict the early outcome after closure of a diverting ileostomy in patients with ileorectal anastomosis since it has been shown that the number of bowel movements after surgery strongly correlated with the anal pressure and the neorectal compliance^[98]. This technique also helped to elucidate better the effects of surgery; in fact, prolonged manometric examination showed that the internal anal sphincter is damaged in the course of mucosal proctectomy and endoanal anastomosis, whereas after restorative proctocolectomy with stapled, end-to-end anastomosis a normal function of the internal sphincter is preserved^[99]. Of note, it has been shown that preservation of the rectoanal inhibitory reflex correlated with a decrease in the incidence of nocturnal soiling after confectioning of a double-stapled ileoanal reservoir^[100]. Thus, several authors have relied on functional assessment to tailor the surgical reconstructive approach in UC patients^[101-105].

CONCLUSION

Although relatively frequent, the co-presence of gastrointestinal motility abnormalities in IBD patients is still relatively unexplored. In fact, looking at the literature data, it is evident that only a few studies are available and most of the more recent literature is focused on the relationship of IBS/IBD^[106]. Thus, information on this topic is still scarce, especially concerning the upper gut. In addition, no controlled data are available regarding the response to treatment of motility changes and/or functional symptoms in IBD.

Moreover, little information is available on whether these motor abnormalities may influence delivery of drugs within the gut in IBD patients since most studies on this topic have been carried out in healthy volunteers. However, some patient data suggest that an increased gastrointestinal transit might impair absorption of several mesalazine formulations^[107]. An accelerated transit might also modify the release of budesonide or multi matrix formulation compounds, but data are lacking^[108]. Finally, some literature data suggest that abnormalities of rectal wall motility might increase mesalazine absorption in IBD patients with ileorectal anastomosis compared to controls^[109].

Hopefully, with the rising interest in the role of low grade inflammation as an important pathophysiological factor in the genesis of several gastrointestinal disorders^[110], more information will be available on the role of neuroimmune interactions leading to symptoms that may complicate the clinical picture in patients with IBD, especially when the major flares of disease are resolved.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Current issues in pediatric inflammatory bowel disease-associated arthropathies

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Abstract

Joint involvement is the most common extraintestinal manifestation in children with inflammatory bowel disease (IBD) and may involve 16%-33% of patients at diagnosis or during follow-up. It is possible to distinguish asymmetrical, transitory and migrating arthritis (pauciarticular and polyarticular) and spondyloarthropathy (SpA). Clinical manifestations can be variable, and peripheral arthritis often occurs before gastrointestinal symptoms develop. The inflammatory intestinal pattern is variable, ranging from sub-clinical inflammation conditions, classified as indeterminate colitis and nodular lymphoid hyperplasia of the ileum, to Crohn's disease or ulcerative colitis. Unlike the axial form, there is an association between gut inflammation and evolution of recurrent peripheral articular disease that coincides with a flare-up of intestinal disease. This finding seems to confirm a key role of intestinal inflammation in the pathogenesis of SpA. An association between genetic background and human leukocyte antigen-B27 status is less common in pediatric than in adult populations. Seronegative sacroiliitis and SpA are the most frequent forms of arthropathy in children with IBD. In pediatric patients, a correct therapeutic approach relies on the use of nonsteroidal antiinflammatory drugs, local steroid in-

jections, physiotherapy and anti-tumor necrosis factor therapy (infliximab). Early diagnosis of these manifestations reduces the risk of progression and complications, and as well as increasing the efficacy of the therapy.

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Key words: Inflammatory bowel disease; Spondyloarthropathy; Sacroiliitis; Infliximab; Extraintestinal manifestations

Core tip: Extraintestinal manifestations in pediatric patients with inflammatory bowel disease (IBD) are very common and are often underestimated, despite provoking a significant impairment of quality of life of patients. This review examines recent literature concerning joint involvement in the course of IBD, focusing on the most important aspects regarding classification of forms of arthropathy, pathogenesis and the essential elements for a correct diagnostic and therapeutic approach.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of unknown etiology characterized by involvement of the gastrointestinal tract and association with certain characteristics, such as familial history, intermittent course, good responsiveness to steroids and high prevalence of extraintestinal manifestations

(EIMs)^[1]. The most common EIMs affect articulations, cutis, eyes and mouth^[2]. The finding of an involvement of different organs and systems leads to the assumption of the presence of predisposing common risk factors. Joint involvement is the most common EIM in children with IBD, which may involve 16%-33% of patients at diagnosis or during follow-up. It is possible to recognize both peripheral and axial forms with a broad clinical spectra, which can be transient and mild to persistent and disabling. In patients with IBD, the diagnosis of arthritis is essentially clinical and radiological; since magnetic resonance imaging (MRI) has become commonly used in clinical practice, it is possible to recognize early forms of articular manifestations^[3].

ARTICULAR INVOLVEMENT IN IBD

Articular-peripheral complications and axial involvement occur in 23% and 4%, respectively, of IBD adult patients^[4]; in this group, 1 out of 5 shows peripheral arthritis, axial arthritis or both^[5,6]. Stawarsky *et al*^[7] carried out an epidemiological study on pediatric IBD patients that confirmed IBD-associated arthropathy in 7%-25% of patients. Although in some studies, there was an increased prevalence of arthritis in the pediatric population compared to adults^[8], with female prevalence. In a recent retrospective, prospective study, the phenotypic expression of the disease between patients with childhood-onset IBD (133 pediatric patients) and adulthood-onset (179 adult patients) cases was evaluated, observing that EIMs in pediatric age patients were more frequent (14.3% *vs* 7.3%) and joint involvement had the same incidence (4.1% *vs* 4.5%)^[9]. Lakatos *et al*^[10] have suggested that in 29% of pediatric IBD there was a risk of developing EIMs within a follow-up period of 15 years. Dotson *et al*^[11] examined the rates of EIMs in a pediatric IBD population, and reported the prevalence of arthralgias (17%), followed by aphthous stomatitis (8%) and arthritis (4%). Furthermore, joint symptoms were correlated with severity and activity of intestinal disease. Orchard *et al*^[12] proposed a classification of enteropathic peripheral arthropathies in adults, distinguishing between Type 1 (pauciarticular, large, inferior articulations) and Type 2 arthritis (polyarticular, small, superior articulations). Type 1 arthritis (4%-17% of patients with Crohn's disease, CD) is correlated with IBD-activity and affects less than five joints (usually ankles, knees, hips, wrists and, sometimes, elbows and shoulders) with evidence of swelling or effusion. Type 2 (2.5% of patients with CD) follows a course independent of the activity of IBD, with persistent symptoms. Type 1 arthritis is more frequent in adult patients with stenosing and penetrating perianal CD, and twice as frequent in patients with colonic and ileocolonic disease, as opposed to patients with ileal disease. Another form of arthritis has been proposed, type 3 peripheral, which includes patients with both axial involvement and peripheral arthritis.

Axial forms are different, with a clinical course gen-

erally independent of IBD activity index, and sacroiliitis (SI) may also be asymptomatic in 50% of patients with CD^[13,14]. In ulcerative colitis (UC), articular complications are more frequent in patients with pancolitis, as opposed to patients with proctitis or left-sided UC^[15].

CLASSIFICATION OF SPONDYLOARTHROPATHIES

Spondyloarthropathies (SpAs) are defined as a group of chronic inflammatory diseases characterized by a pauciarticular spondyloarthropathy, peripheral, or asymmetrical arthritis, with or without axial involvement^[16]. SpAs are considered a variety of EIMs of IBD, and the clinical features, which are initially not well defined, evolve over time, becoming clearer at a later stage. The classification proposed by Amor *et al*^[17] and Dougados *et al*^[18] includes clinical entities such as axial forms; SI; ankylosing spondylitis (AS); reactive arthritis (ReA), which usually occurs because of infective enteritis, especially *Salmonella*, *Shigella*, *Yersinia* or *Campylobacter*; peripheral arthritis, usually asymmetrical, affecting main articulations; psoriatic arthritis; seronegative enthesopathy and arthropathy syndrome and enthesitis; uveitis and IBD-associated arthropathy (Table 1). To group all forms of arthritis that begin before the age of 16 years, experts in pediatric rheumatology, the International League of Associations for Rheumatology^[19], have developed a classification of juvenile idiopathic arthritis (JIA), revised in 2004, which includes seven subgroups: (1) systemic arthritis; (2) oligoarthritis; (3) polyarthritis (rheumatoid factor negative); (4) polyarthritis (rheumatoid factor positive); (5) enthesitis related arthritis; (6) psoriatic arthritis; and (7) undifferentiated arthritis (originally called "other arthritis"). In this classification, in contrast to the European Spondyloarthropathy Study Group (ESSG) criteria, there is the exclusion of children with a family history of psoriasis, and the absence of ReA and IBD as forms of SpA. IBD appears only as a descriptor of enthesitis related arthritis forms, but the term is not defined, even if UC and CD can clearly be associated with human leukocyte antigen (HLA)-B27, undifferentiated SpA, or even AS^[20,21].

The most commonly accepted classification criteria for SpAs, used in clinical practice, come from the ESSG (Table 1). These criteria are superior to the earlier New York modified criteria for the diagnosis of AS (Table 1), but are inadequate in describing the full clinical peculiarities of SpAs, as they exclude some manifestations^[22] (Table 1). Furthermore, this classification has not proved very useful in defining patients with forms of early arthritis (*e.g.*, less than 1 year of onset); in fact, it was drawn up before MRI became commonly used in clinical practice. To overcome these defects, in particular to enable diagnosis before the appearance of radiological damage, other classifications have been validated. The most recent is the Assessment of SpondyloArthritis International Society criteria for the classification of axial^[23,24] and peripheral spondyloarthritis^[25]. They are an innova-

Table 1 Classification of spondyloarthropathies

Spondyloarthropathies: Five major subtypes
Ankylosing spondylitis (AS)
Reactive arthritis (ReA)
Psoriatic arthritis
Enteropathic arthritis or arthritis associated with inflammatory bowel disease
Undifferentiated spondyloarthropathy
European spondyloarthropathy study group
Classification criteria for spondyloarthropathy
Inflammatory spinal pain OR synovitis (asymmetrical or predominantly in the lower limbs) plus any one or more of the following:
Positive family history
Alternate buttock pain
Psoriasis
Enthesopathy
Inflammatory bowel disease
Sacroiliitis
Modified New York criteria for the diagnosis of ankylosing spondylitis
Unilateral sacroiliitis grade 3 or 4, or bilateral sacroiliitis grade 2 to 4 together with at least one of the following:
Low back pain of at least three months' duration improved by exercise and not relieved by rest
Limited motion of lumbar spine in sagittal and frontal planes
Decreased chest expansion relative to normal values for age and sex
Musculoskeletal manifestations of spondylarthropathies
Peripheral arthritis: one or more swollen and tender joint (s); synovitis is asymmetric and predominantly in lower limbs
Inflammatory spinal pain: symptoms of back pain in lumbar, dorsal or cervical regions associated with at least four of the following: (Calin's criteria)
Onset before age 45 yr
Insidious onset
Improved by exercise
Associated with morning stiffness
Duration of at least three months
Dactylitis: evidence of "sausage digit" on examination
Peripheral enthesitis: achilles tendinitis and/or plantar fasciitis
Buttock pain
Anterior chest wall pain

tion compared to the previous criteria (ESSG and Amor *et al.*^[17]), but still have limitations in clinical practice, especially if applied in a pediatric population^[26]. Although Amor's and the ESSG criteria were derived from a population of adult patients with SpA, they might be applied specifically in juvenile onset SpA.

PATHOGENESIS

Factors that determine the presence of SI and AS remain unclear; however, HLA-B27 typing may be a potential factor. The frequency of HLA B27 in the IBD population is generally not higher compared with the general population. HLA-B27 confers an additional risk for inflammatory low back pain in patients with IBD^[27]. A prospective study concerning genetic variability in these populations has found a significantly higher rate of HLA B27 in patients with IBD and AS compared with those with undifferentiated SI. HLA B27 can detect 60%-90% of patients with SI diagnosed at a young age who subsequently develop AS^[28]. Another candidate gene, CARD 15, is described in association with CD^[29], but studies have not demonstrated any association with extra-intestinal involvement. De Vos *et al.*^[30] found a striking association between a CARD 15 polymorphism and SI (78% in patients with SI *vs* 48% without SI). No studies have been carried out on the association of CARD 15 and SpA in children with CD. In conclusion, seronegative

SI and SpA are the most frequent forms of arthropathy in children with IBD, especially in CD. In these patients, it may be useful to carry out a screening with Rx of the lombo-sacral rachis, also in the absence of typical symptoms. A transmembrane glycoprotein, E-cadherin, has been identified in the literature. It mediates the intercellular adhesion of epithelial cells, which, for its role of receptor for CD103, is considered a marker of monocyte-derived inflammatory dendritic cells that may contribute to the pathogenesis of chronic T cell-mediated colitis^[31]. Demetter *et al.*^[32] showed that expression of E-cadherin is high in the gut of patients with IBD and in patients with SpA^[33]. Moreover, this integrin was found in subclinical acute and chronic gut inflammation, as an early event in the development of this process. Other alterations common in the two groups concern CD4⁺ T cells (Th1 cells, Th2 cells, Th17 cells and regulatory T cells). Initial studies have shown Th1 predominance in the intestinal mucosa of patients with IBD and SpA. Recent studies have suggested that, in both groups of patients, Th17 cells may have an important role in the initiation and perpetuation of autoimmune inflammation^[34]. Many studies have established an important role of toll-like receptors (TLRs) in the innate immune response against pathogenic microorganisms. Several studies have shown increased expression of TLR-4 and TLR-2 in APCs of patients with SpA.

Immunopathological overlap between gut inflamma-

tion and SpA has been demonstrated. Intestinal inflammation is believed to play a key role in the pathogenesis of SpA^[35]. It has been hypothesized in some studies that ileocecal inflammation may be considered an important risk factor in the occurrence of SI. Ileocolonscopies performed in adults with AS, without clinical intestinal symptoms, have shown inflammatory infiltrate of the ileum mucosa in 65% of subjects examined compared with 3% in a control group^[36]. Studies carried out on patients with SpA have exhibited ileal and colonic inflammatory infiltrate in the lamina propria, with signs of architectural distortions^[37]. Mielants *et al*^[38], in another study on adult patients with AS, demonstrated an inflammatory gut histological pattern with “acute” inflammatory infiltrations, characterized by a well preserved architecture with neutrophil- and eosinophil-like epithelial infiltrate, with no significant lymphocyte infiltrate. This “chronic” pattern, with histological alterations similar to those in IBD (crypt distortion and/or atrophy, cryptitis, and/or crypt with increased rate of mixed cells and lymphoid aggregates in the lamina propria), was classified as indeterminate colitis (IC)^[39] and was less frequent. Subclinical intestinal lesions are most frequent in a pediatric population (75% of cases) with undifferentiated SpA (peripheral arthritis); in 16% of cases (2/12), CD occurred during follow-up^[40].

Conti *et al*^[41], in 129 children with SpA and clinical symptoms suggestive for IBD, used ileocolonoscopy to confirm a diagnosis of IBD only in seven (5%), while 12 (9%) had IC, and in 12 (9%) patients, intestinal nodular lymphatic hyperplasia was present as a main characteristic. Subclinical intestinal inflammation in patients with SpA is frequent in pediatric populations, and a long-term follow-up may be needed to determine if IC and intestinal nodular lymphatic hyperplasia can be considered as early forms of IBD.

DIAGNOSTIC APPROACH

In patients with IBD, the diagnosis of arthritis, both peripheral and axial, is essentially clinical and radiological, as there is no reliable laboratory test that can be used as a diagnostic tool for diagnosis and management of these arthropathies^[42].

Early diagnosis of inflammatory arthritis in IBD patients may prevent disability caused by SpA and AS^[43]. Lakatos *et al*^[44] reported SI as the most frequent arthropathy in CD (20%) compared to UC (15%) and predominantly in females, especially in children. SI is clinically characterized by lower back pain and involves the lumbosacral region with functionally impaired rachis. The gold standard for diagnosis is a radiography (Rx) of the lumbosacral rachis following the radiological criteria expressed in the modified New York classification^[45,46]. Orchard *et al*^[47] compared MRI with the gold standard in adult patients, adapting the New York criteria to MRI of the rachis: the results obtained with the latter imaging technique were much more accurate and specific. Their study, carried out

on a population of patients with CD, revealed that about 39% of patients showed signs of SI detected by MRI, while previous studies showed signs of SI in 20% of IBD patients diagnosed with Rx^[48].

SpAs associated with IBD are placed in differential diagnosis with other pathologies with osteo-articular involvement, including osteoarthritis, rheumatoid arthritis and arthritis associated with connective tissue diseases, such as lupus, arthralgia (which may complicate corticosteroid withdrawal), osteonecrosis related to corticosteroids, and infliximab (IFX) related lupus-like syndrome.

However, in many cases, intestinal involvement is underestimated in patients with SpA, because these patients often have no intestinal symptoms and high levels of blood markers of inflammation are erroneously attributed only to the joint disease. For this reason, in clinical practice, it may be necessary to use noninvasive investigations on the gut for a preliminary assessment of patients with suspected intestinal disease. Serum levels of human cartilage glycoprotein 39 (also called YKL-40) were recently found to be higher than normal in IBD patients with arthropathies, suggesting that this protein could be used as a disease activity marker in arthritis associated with IBD. Moreover, we can use certain leukocyte proteins, such as lactoferrin and calprotectin, as markers of the presence of leukocytes in stools, to assess intestinal inflammation. Serological tests focus on several antibodies, such as perinuclear anti-neutrophil cytoplasmic antibodies and anti-Saccharomyces cerevisiae antibodies, used in combination to increase sensitivity. Additional serum biomarkers include antibodies against outer membrane porin C, the pseudomonas fluorescens bacterial sequence I2, bacterial flagellin and the anti-glycan antibodies, *i.e.*, anti-chitobioside IgA, anti-laminaribioside IgG and antimannobioside^[49].

Other non-invasive methods of screening patients presenting arthropathies where intestinal involvement is suspected, include abdominal ultrasound to visualize bowel wall thickness and capsule endoscopy of the small intestine to detect intestinal inflammation before performing endoscopic evaluation^[50].

THERAPY

Treatment of arthritis and arthropathy associated with IBD is based almost entirely on extrapolation from therapy for other forms of arthritis. The therapeutic approach in IBD-associated arthropathies must be oriented towards treatment of the prevalent disease (articular *vs* intestinal disease) with the identification the best approach. Conventional treatment depends on clinical presentation (axial disease, peripheral arthritis, enthesopathy) or associated features (uveitis, psoriasis, colitis). Lee *et al*^[51] have proposed a therapeutic algorithm for patients with SpA indicating specific anti-inflammatory molecules, such as nonsteroidal anti inflammatory drugs (NSAIDs) and cyclooxygenase-2 (COX-2), for axial and peripheral arthropathies, in a 1st level treatment, while

Table 2 European Crohn's and colitis organization recommendations

Peripheral arthritis: Short term treatment with non-steroidal anti-inflammatory agents, local steroid injections and physiotherapy (primary focus for underlying Crohn's disease)
Persistent peripheral arthritis: Sulfasalazine
Axial arthropathy: Intensive physiotherapy associated with non steroidal anti inflammatory drugs
Ankylosing spondylitis and Crohn's disease intolerant or refractory to non steroidal anti inflammatory drugs: Anti-tumour necrosis factor therapy

sulphasalazine, mesalazine or methotrexate (MTX) were recommended in moderate peripheral arthritis, in addition to intra-articular steroid injections and physiotherapy. NSAIDs may worsen an existing colitis^[52], although it has also been observed that therapies with COX2 inhibitors, which last up to 2 wk, have not induced any relapses in patients with UC^[53]. In IBD-associated arthropathies, MTX, azathioprine or Sulphasalazine are considered to be ineffective or marginally effective if axial symptoms predominate, as opposed to peripheral arthritis^[54-56]. The two main biological agents targeting tumor necrosis factor (TNF)- α are the chimeric monoclonal IgG1 antibody (IFX), and the 75 kD IgG1 fusion protein (Etanercept)^[57]. Several trials have confirmed the effectiveness of IFX, especially in AS^[58,59]. IFX and etanercept are also effective in the treatment of rheumatic symptoms, such as peripheral arthritis and SI in IBD patients^[60]. Few data are available regarding the efficacy of etanercept in pediatric patients with IBD-associated arthritis^[61]. Sandborn *et al*^[62] confirmed the inefficacy of Etanercept on control of intestinal disease in patients with SI or AS, and IBD. It was demonstrated that Etanercept, despite controlling the musculoskeletal features associated with arthritis, and in particular enthesal, is not effective in controlling colitis in patients with Crohn's SpA, suggesting that the effect of TNF- α blockade in SpA differs between the joint and the bowel^[63]. On the other hand, IFX has proven effective in inducing and maintaining endoscopic and clinical remission in pediatric CD^[64]. Brandt *et al*^[65] reported beneficial effects of IFX on axial and peripheral symptoms in patients with CD and Kaufman *et al*^[66] confirmed the efficacy of IFX in EIMs of CD. Ellman *et al*^[67] described four patients with CD arthritis where IFX has induced clinical improvement with reduced need for corticosteroids. Generini *et al*^[68] have proposed a therapeutic flow-chart in SpA associated with CD, with the use of IFX at a dose of 5 mg/kg. A maintenance schedule provided infusions every 5-8 wk, and results showed clinical remission in both intestinal and articular diseases for 12-18 mo. IFX appears to be the drug of choice for treating both pathologies.

Braun *et al*^[69], in a multicenter randomized trial, demonstrated the persistent clinical efficacy and safety of anti-TNF therapy with IFX (5 mg/kg) in patients with AS over 5 years of almost continuous treatment, with low rates of drug-related adverse events.

Current ECCO recommendations suggest the use of short-term treatment with NSAIDs, local steroid injections and physiotherapy in CD patients with arthropathies with better control of the intestinal disease. In the

case of axial arthropathy, NSAIDs are considered first-line drugs, but in the case of refractory or intolerant forms to NSAIDs, anti-TNF agents are recommended^[70] (Table 2).

Adalimumab (ADA) is effective at inducing and maintaining remission in children with CD, and is effective for UC patients with loss of response or with adverse effects secondary to IFX^[71,72]. ADA has also been studied in pediatric patients with rheumatic diseases, with demonstrated efficacy in pediatric patients with JIA^[73] and ERA^[74]. However, data relating to ADA use in pediatric patients with joints disease associated with IBD are scarce.

CONCLUSION

IBD, which includes CD and UC, can be a painful and debilitating condition. In addition to bowel symptoms, patients with IBD often experience extraintestinal complications, such as arthritis, kidney and liver disease, eye disorders and skin problems. Of these, arthritis is the most common - occurring in about 25% of all pediatric IBD sufferers. Evidence indicates that a dysregulation of mucosal immunity in the gut of IBD causes an overproduction of inflammatory cytokines into the bowel, thus leading to an uncontrolled intestinal inflammation with joints involvement. TLR variants and abnormalities, altered function and balance of T-cell subpopulations and their production of proinflammatory cytokines, as well as integrin and E-cadherin dysfunction, are partially responsible for the pathogenesis of these complex diseases. Medical treatment of rheumatic manifestations of IBD includes sulfasalazine and mesalamine, immunomodulators and TNF- α inhibitors. As our understanding of the pathophysiology of ERA, enthesitis, and sacroiliitis improves, and with the advent of additional biological therapies, the prognosis of children and adolescents with arthropathies associated with IBD will likely improve.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Inflammatory bowel disease: Epidemiology, pathology and risk factors for hypercoagulability

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Abstract

Hypercoagulability observed in patients with inflammatory bowel diseases (IBD) may lead to thromboembolic events (TE), which affect the venous and arterial systems alike and are an important factor in patients' morbidity and mortality. The risk of TE in IBD patients has been demonstrated to be approximately three-fold higher as compared to the general population. The pathogenesis of thrombosis in IBD patients is multifactorial and not fully explained. The most commonly listed factors include genetic and immune abnormalities, disequilibrium between procoagulant and anticoagulant factors, although recently, the role of endothelial damage as an IBD-triggering factor is underlined. Several studies report that the levels of some coagulation enzymes, including fibrinogen, factors V, VII, VIII, active factor XI, tissue factor, prothrombin fragment 1 + 2 and the thrombin-antithrombin complex, are altered in

IBD patients. It has been demonstrated that there is a significant decrease of tissue plasminogen activator level, a marked increase of plasminogen activator inhibitor type 1 and thrombin-activable fibrinolysis inhibitor, a significantly lower level of antithrombin III and tissue factor pathway inhibitor. IBD patients have been also observed to produce an increased amount of various anticoagulant antibodies. Hyperhomocysteinemia, which is a potential risk factor for TE was also observed in some IBD patients. Further studies are necessary to assess the role of coagulation abnormalities in IBD etiology and to determine indications for thromboprophylactic treatment in patients at high risk of developing TE.

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Key words: Crohn's disease; Hypercoagulation; Risk factors; Thrombosis; Ulcerative colitis

Core tip: Thromboembolic events (TE) in inflammatory bowel diseases (IBD) patients are often overlooked. They affect both the venous and arterial systems. The inflammatory process initiates clotting, impairs the fibrinolytic system and decreases the activity of natural anticoagulation mechanisms. Depression of anticoagulation mechanisms not only increases thrombosis, but also potentiates the inflammatory process. The objective of the present report is to demonstrate the high significance of a problem posed by hypercoagulability in IBD patients based on TE epidemiology, and to present abnormalities in the hemostatic system.

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INTRODUCTION

Inflammatory bowel diseases which include Crohn's disease (CD) and ulcerative colitis (UC), are systemic disorders that predominantly affect the gastrointestinal tract, but are also associated with a number of extraintestinal manifestations. The majority of extraintestinal complications are characterized by less intense symptoms as compared to those associated with the primary disease, but thromboembolic events (TE) may increase mortality rates in inflammatory bowel diseases (IBD)^[1-3]. TE in IBD are often overlooked, since their detection rate in IBD patients is only 6% as compared amounts approximately to 40 % in postmortem examinations^[4-6].

The pathogenesis of thrombosis in IBD patients is multifactorial and not fully explained. Numerous investigations have demonstrated qualitative and quantitative abnormalities in procoagulation, anticoagulation and fibrinolytic factors that predispose to thrombosis development in IBD, although some authors have not found any risk factors in about one-half of IBD patients diagnosed with thrombosis.

IBD is a result of an interaction of acquired and genetic factors, with the very inflammatory process being of high significance as well^[1,2]. The inflammatory process initiates clotting, impairs the fibrinolytic system and decreases the activity of natural anticoagulation mechanisms; on the other hand, natural anticoagulation factors reduce elevated levels of cytokines triggered by inflammation^[7]. Thus, depression of anticoagulation mechanisms not only increases thrombosis, but also potentiates the inflammatory process. An example of the effect of procoagulation factors on the inflammatory process may be found in thrombin, which increases the production of tumor necrosis factor, interleukin (IL)-6 and IL-10 by signaling protease-activated receptors and, therefore, is able to amplify and modify inflammation^[8].

The objective of the present report is to demonstrate the high significance of a problem posed by hypercoagulability in IBD patients based on TE epidemiology, and to present abnormalities in the hemostatic system.

EPIDEMIOLOGY OF TE IN IBD PATIENTS

TE are among complications demonstrated in IBD patients, which affect the venous and arterial systems alike and are an important factor in patients' morbidity and mortality. The risk of systemic TE development in IBD patients has been demonstrated to be approximately three-fold higher as compared to the general population^[1,2]. TE in IBD has been also demonstrated to develop in younger individuals as compared to the general population^[9].

The incidence of TE in IBD has been estimated to be approximately 0.1%-0.5% per year, with an overall mortality rate as high as 25% per episode^[2]. In clinical studies, the incidence rate of TE in IBD patients is estimated as falling in the range of 1.3%-7.7%^[1,4,5], although the rate

increases to 39%-41% in postmortem examinations^[1,6]. For this reason, based on clinical studies published to date, one may say that systemic TE in patients with CD and UC are underdiagnosed.

TE occur mainly during disease exacerbation and are more common in IBD patients with markedly elevated inflammatory markers and presenting with other complications, such as strictures, fistulisation, or abscesses^[1,5,10]. The incidence of such episodes is also correlated with the extent of the disease, especially in pancolonic UC patients and in CD patients with colonic involvement^[11,12]. However, proctocolectomy is not protective of recurrent deep venous thrombosis (DVT) or pulmonary embolism (PE)^[11]. While studying the frequency of TE recurrence in IBD patients in several-year follow-up, an increased risk of venous thromboembolism (VTE) was demonstrated as compared to patients without IBD^[11,13]. In addition, the risk is three times higher in males as compared to females^[13]. In the majority of women, pregnancy is uncomplicated^[14]. No higher risk of TE was observed in pregnant women^[15].

The age at first VTE is significantly associated with an increased risk of recurrence^[13]. The study assessing the risk of TE in the population of Danish children with IBD showed that relative risks were higher in patients under 20 years of age, though actual incidence increased with age^[16]. A higher incidence of cerebral TE in pediatric population with IBD was noticed, as well^[17].

When compared to general outpatient population, the risk of VTE is 16 times higher in IBD patients who are not hospitalized during the active phase of the disease^[18]. However, it is important that thrombosis is also reported in patients with well-controlled disease^[5,18]. It has been suggested that in contrast to CD, UC is associated with an increased risk of TE in patients with low-activity disease or even during remission^[19,20].

The most common TE in IBD are lower extremity DVT and PE^[21]. Occasionally, VTE occur in the cerebral, hepatic, portal, retinal, and mesenteric veins^[21-23]. Arterial TE occur less frequently than VTE; the former include thrombosis of the cerebral and retinal arteries and also arteries of upper and lower limbs^[23-25]. Moreover, there have been reports of cases of coronary artery thrombosis in young patients with IBD^[26].

It is important to remember that in IBD, thrombosis involves not only the systemic veins and arteries, microthrombi may involve the vasculature of the uninfamed intestine as well^[27]. For instance, in patients with CD platelet thrombi cross-linked with fibrin were demonstrated in the mucosal microvasculature^[28], in patients with UC intracapillary clots have been observed in rectal biopsies^[29]. Several observational studies have shown a potential benefit of using heparin treatment in patients with IBD^[30,31].

Very interesting results have been presented by Thompson *et al*^[32]. The authors have observed that in patients with inherited bleeding disorders such as hemophilia A, hemophilia B and von Willebrand disease, the

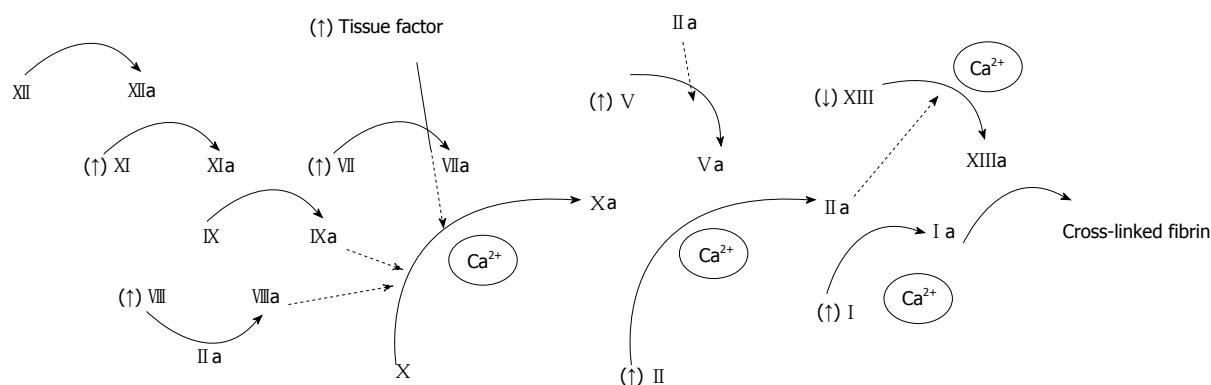


Figure 1 Coagulation cascade model. ↑/↓: Changes in coagulation factors, in patients with inflammatory bowel diseases.

Table 1 Acquired prothrombotic factors in inflammatory bowel diseases

Dehydration
Glucocorticosteroids therapy
Prolonged immobilization
Central venous catheters
Surgical procedures
Oral contraceptives/hormonal replacement therapy
Smoking
Hyperhomocysteinemia

risk of development of either CD or UC was significantly decreased.

It has been suggested that hypercoagulability, prothrombotic state and vascular occlusion play an important role in the pathogenesis of IBD.

ETIOLOGY OF THROMBOSIS IN IBD

IBD patients demonstrate disequilibrium between pro-coagulant and anticoagulant factors, which predisposes them to develop thrombosis; the abnormalities are both quantitative and qualitative^[33].

Acquired factors that affect disturbances in the hemostatic system in IBD include prolonged immobilization, surgical procedures, central venous catheters, glucocorticosteroids therapy, oral contraceptives, hormonal replacement therapy, cigarette smoking, hyperhomocysteinemia, vitamin deficiency, dehydration, as well as damage to the vascular endothelium^[21,34,35] (Table 1). Hypercoagulability in both CD and UC may be also triggered by genetic factors^[20].

The hemostatic system is an indispensable element of each inflammatory process. During the inflammatory process, not only proteases originating from inflammatory cells are activated, but also those originating from the coagulation and fibrinolysis system^[1,36]. For this reason, an increased risk of TE complications is also present during remission period - mostly in patients with UC. The phenomenon is most likely related to the interaction between cytokine mediators of chronic inflammation and the coagulation cascade^[34].

The mechanism of an increased thromboembolic risk in IBD is complex, multifactorial and not fully understood. It appears to be multifactorial because no consistent unifying etiology has been identified. However, there are also studies available, where in approximately one-half of IBD patients who developed TE no possible causative factors associated with the complications have been identified^[37].

Coagulation cascade

The coagulation cascade is essentially a series of enzymatic conversions, turning inactive proenzymes into activated enzymes and culminating in the formation of thrombin. Thrombin then converts the soluble plasma protein - fibrinogen - precursor into the insoluble fibrous protein - fibrin. Each reaction in the pathway results from the assembly of a complex composed of an enzyme (an activated coagulation factor), a substrate (a proenzyme form of a coagulation factor) and a cofactor (a reaction accelerator).

In the classical cascade model, coagulation has been divided into the extrinsic and intrinsic pathway, converging at the point where factor X (FX) is activated (Figure 1).

Several studies report that the levels of some coagulation enzymes are altered in IBD patients, including increased fibrinogen, increased factor V (FV), VII (FVII) and VIII (FVIII) and also increased prothrombin fragment 1 + 2 and the thrombin-antithrombin complex^[21,34]. The increase in the level of these coagulation factors in CD and UC patients is associated with disease activity^[34,38]. Elevated levels of circulating active factor XI (FXI) and tissue factor (TF) have been also reported in IBD^[39]. Another abnormality in the coagulation cascade in IBD patients is a decrease in the level of factor XIII (FXIII)^[34,40-44]. In the majority of publications a decrease of FXIII level was described in patients with active phase of CD and UC in comparison to non-active phase^[34,41,42,44]. There is also a research that does not demonstrate any correlation between disease activity and a FXIII level^[40]. One of the potential causes of reduced activated FXIII plasma levels in IBD might be its consumption in the repair of injured tissue^[40,41,43] or in the increased formation of microthrombi^[42].

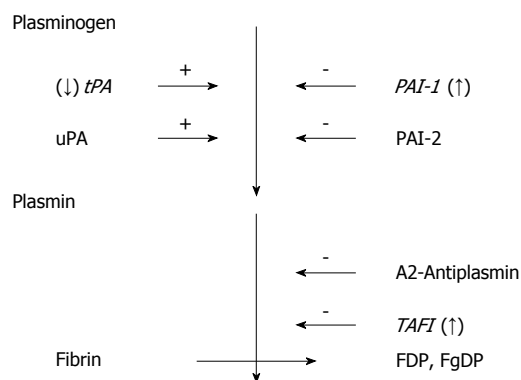


Figure 2 The elements of the fibrinolytic system. tPA: Tissue-type plasminogen activator; uPA: Urokinase plasminogen activator; PAI-1: Plasminogen activator inhibitor type 1; TAFI: Thrombin activatable fibrinolysis inhibitor; FDP: Fibrin degradation products; FgDP: Fibrinogen degradation products. ↑/↓: Changes in coagulation factors, in patients with inflammatory bowel diseases.

Anticoagulation mechanisms

Anticoagulation mechanisms, often referred to by a simplified term “anticoagulation system” are responsible for balancing the procoagulation tendency in the hemostatic system. The system includes the fibrinolysis system, plasma coagulation inhibitors, protein C (PC) anticoagulant system and vascular wall.

Fibrinolysis system

The basic element of the fibrinolysis system is plasminogen, which - following its transformation by activators to an active substance, *i.e.*, plasmin, acts upon fibrinogen, fibrin, FV, FVII, FXIII, von Willebrand factor and platelet glycoproteins.

The elements of the fibrinolysis system - both activators and inhibitors - are presented in Figure 2.

Disturbances in the fibrinolysis system are another very important factor in IBD patients that is associated with hypercoagulability. The fibrinolytic system has been widely investigated in patients with CD and UC, and hypofibrinolysis has been described as a potential contributor to the hypercoagulable state in IBD patients^[21,34].

In IBD patients, there has been demonstrated a significant decrease of tissue plasminogen activator (tPA) level, the principal activator of the fibrinolysis system, as compared with the controls^[34,45]. With respect to inhibitors of the fibrinolysis system, IBD has been shown to demonstrate a marked increase of plasminogen activator inhibitor 1 (PAI-1) and thrombin-activatable fibrinolysis inhibitor (TAFI) levels as compared to the healthy control subjects^[45,46].

TAFI is a modulator of homeostasis that provides a link between the coagulation system and fibrinolysis system, as well as between the hemostatic system and the inflammatory process^[46].

TAFI, a plasma zymogen, can be activated by thrombin, the thrombin-thrombomodulin complex, or plasmin. The activated form of TAFI (TAFIa) removes C-terminal lysine residues from plasmin-modified fibrin which sup-

presses plasminogen activation, thereby attenuating fibrinolysis. In addition to suppressing fibrinolysis, TAFIa may also be involved in inflammation. Its potential role as a natural anti-inflammatory molecule is currently being explored, with recognition of its ability to inactivate potent anaphylatoxins, C3a and C5a, as well as the proinflammatory mediators, bradykinin and osteopontin^[45,46]. TAFI has been also demonstrated to be associated not only with development of thromboembolic complications in various disease entities, but also to affect the course of such diseases^[46]. In IBD, a significant correlation has been demonstrated between TAFIa and such inflammatory markers as CRP, fibrinogen, platelets, as well as disease activity^[46].

Plasma coagulation inhibitors

Figure 3 presents plasma coagulation inhibitors.

Antithrombin III (ATIII) is the best known natural coagulation inhibitor, its activation is triggered by heparin secreted by mast cells or administered exogenously. ATIII is predominantly synthesized in the liver, vascular endothelial cells, megakaryocytes and platelets. It is the most important endogenous thrombin inhibitor; it forms complex with thrombin in a 1:1 molar ratio, which is subsequently removed from blood by macrophages. ATIII also inactivates factor Xa, factor XIIa, factor XIa and factor IXa. In addition to anticoagulation properties, ATIII has been demonstrated to have anti-inflammatory properties^[32]. ATIII decreases expression of CD11b/CD18 cell surface receptors on leukocytes, which decreases leukocyte adhesion, decreases expression of tissue factor and IL-6 in monocytes and endothelium. ATIII also increases prostacyclin formation and decreases nuclear factor (NFκB)^[36]. IBD patients have been demonstrated to have a significantly lower levels of ATIII and TF pathway inhibitor (TFPI) as compared to the controls^[8,21,34].

TFPI is a protein produced by vascular endothelial cells and megakaryocytes. It is a Kunitz-type protein with three inhibitor domains: the first domain inhibits the factor VIIa/tissue factor complex, the second binds active factor X, the third is responsible for binding TFPI with heparin and lipoproteins^[36]. TFPI is a total inhibitor of the TF-dependent coagulation cascade and a marker of endothelial damage^[36].

Changes in coagulation and fibrynolytic systems are presented in Table 2.

PC system

The PC system is the most important element of anticoagulation mechanisms. It is presented in Figure 4.

The PC pathway is activated by thrombin bound to endothelial thrombomodulin (TM). Following binding with TM, thrombin acquires abilities to activate PC. PC activation is facilitated when PC is bound to the endothelial cell PC receptor (EPCR). In turn, APC in the presence of protein S (PS) cofactor inactivates active FVIII and FV^[7,36]. Additionally, APC has profibrinolytic properties, since it binds PAI-1^[7,36].

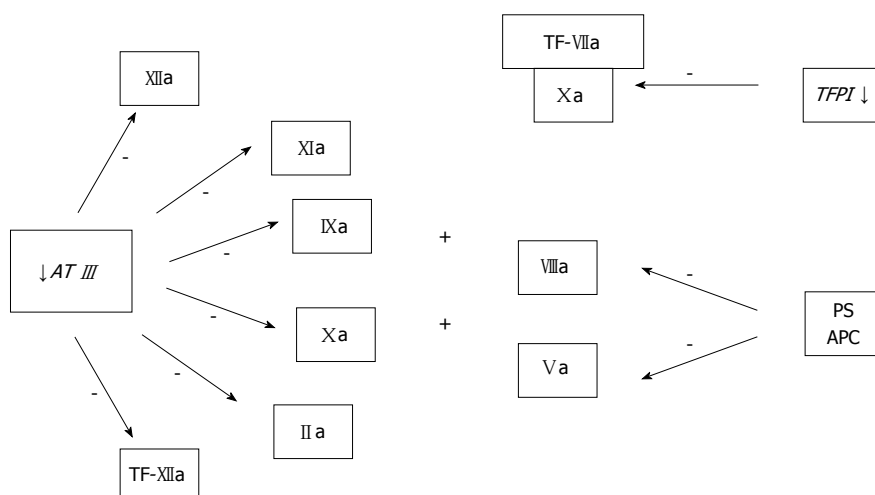


Figure 3 Plasma coagulation inhibitors. AT III: Antithrombin III; TF: Tissue factor; TFPI: TF pathway inhibitor; PS: Protein S; APC: Activated protein C. ↓: Changes in plasma coagulation inhibitors, in patients with inflammatory bowel diseases.

Table 2 Changes in coagulation and fibrinolytic systems in patients with inflammatory bowel diseases

Coagulation factors	Fibrinolytic factors	Plasma coagulation inhibitors
Fibrinogen ↑	TAFI ↑	Antithrombin III ↓
Factor V ↑	PAI-1 ↑	TFPI ↓
Factor VII ↑	tPA ↓	
Factor VIII ↑		
Factor XI ↑		
Prothrombin fragment 1 + 2 ↑		
Thrombin-antithrombin complex ↑		
TF ↑		
FXIII ↓		

tPA: Tissue plasminogen activator; TAFI: Thrombin-activatable fibrinolysis inhibitor; PAI-1: Plasminogen activator inhibitor 1; TFPI: Tissue factor pathway inhibitor.

Studies have demonstrated that TM as well as APC, in addition to exhibiting anticoagulation properties, also affect the course of the inflammatory process, apoptosis and endothelial barrier^[36,47]. Reports of studies on PC, PS and TM levels in patients with CD and UC are contradictory^[18,48], although some investigators have demonstrated PC and/or PS deficiency during active IBD phase^[21,34].

The available reports regarding plasma TM concentrations in IBD patients have also yielded conflicting results. Several studies have demonstrated that higher TM concentrations occur only in active CD patients as compared to the controls; the above authors have failed to demonstrate any changes in PC and PS levels in IBD. In another study, TM concentrations were higher in UC than in the healthy controls^[47].

The controversial character of these findings may result from differences in IBD populations studied (demographic and clinical features, definition of disease activity and evaluation)^[47].

It may be also suggested that increased TM or PC

levels described by some authors may be associated with their anti-inflammatory properties^[47].

Further studies assessing anti-inflammatory properties of TM and PC in IBD are necessary.

Vascular wall

Of importance in regulation of hemostatic mechanisms are subsequent layers of vascular wall: smooth muscles - vasospasm, subendothelial layer - activation of the hemostatic system following endothelial damage, platelet activation, and endothelium.

Hemostatic activity of the endothelium under physiological conditions consists of ensuring non-clotting of blood, non-thrombogenicity of vascular surface, and - after endothelial damage - limiting the thrombus to the thrombogenic site. Endothelial damage disturbs the multifactorial equilibrium provided by endothelial cells, causing development of numerous significant pathological sequelae, such as thrombi formation, hypertension, atherosclerotic lesions, disturbances in tissue perfusion, angiogenesis and inflammatory infiltration^[49].

Endothelial damage occurring in numerous diseases is evident though increasing levels of endothelial injury markers. The most frequently used biochemical markers of endothelial damage include von Willebrand factor (vWF), TM, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and endothelin-1 (ET-1).

In recent years, asymmetric dimethylarginine (ADMA) has been also classified as an endothelial damage marker. It is synthesized during the methylation of protein arginine residues by protein arginine methyltransferases and released during proteolysis. ADMA is a major endogenous NO synthase inhibitor and a competitive inhibitor of the cellular L-arginine uptake^[50]. Elevation of ADMA induces dysfunction of the endothelium, which becomes clinically evident by impaired endothelium-dependent vasodilation, hyperaggregability of platelets and enhanced monocyte adhesion^[50].

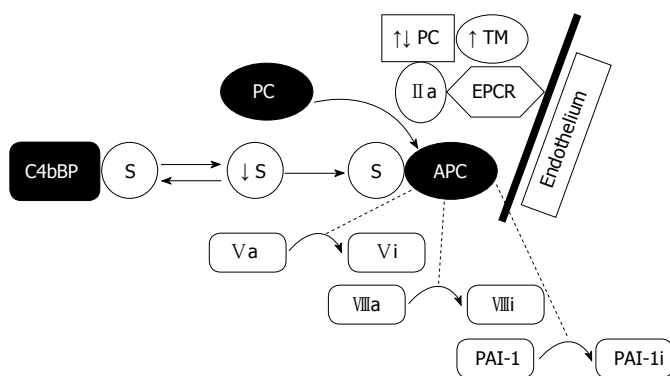


Figure 4 Protein C system. PC: Protein C; APC: Activated protein C; S: Protein S; TM: Thrombomodulin; PAI-1: Plasminogen activator inhibitor 1; EPCR: Endothelial cell PC receptor; C4bBP: C4b-binding protein; Va: V active; Vi: V inactive; VIIIa: VIII active; VIIIi: VIII inactive. ↑/↓: Changes in protein C system, in patients with inflammatory bowel diseases.

According to the published studies, there has been observed an increase of such endothelial damage markers as vWF, TM, endothelial PC receptor (EPCR) and ADMA in IBD patients; they have been also found to correlate with disease activity and inflammatory markers^[34,46,47,50].

Vascular endothelial damage is believed to be a factor that increases the risk of TE in IBD.

Homocysteine

Homocysteine is an amino acid formed from S-adenosylmethionine (SAM), in all types of cells in the human body. It participates in metabolism of numerous compounds that are indispensable for the organism to function.

Homocysteine is a pro-coagulation factor, since it evokes changes in vascular walls and triggers increased expression of TF and FV, decreased expression of TM and tPA and decreased activation of PC. Elevated plasma homocysteine levels facilitate development of both arterial and venous thrombosis^[34]. Recently, homocysteine has been also shown to participate in microvascular inflammation in IBD, triggering endothelial inflammation, resulting in VCAM-1 upregulation, monocyte chemoattractant protein-1 (MCP-1) production and p38 phosphorylation. Such changes lead to increased adhesion of T cells and monocytes to the endothelium^[51].

Hyperhomocysteinemia is a potential risk factor for TE in IBD patients and can be secondary to folic acid and vitamin B12 deficiencies, medications (methotrexate, sulphalazine, glucocorticosteroids), smoking and nutritional deficiencies^[21,34].

Both plasma and mucosal homocysteine levels have been demonstrated to be significantly higher in IBD patients as compared to the healthy controls and correlated with disease activity^[51,52]. However, there is no evidence that an increase in homocysteine level is of greater proportion in IBD patients with TE *vs* those without. The risk assessment of hyperhomocysteinemia-related thrombosis in IBD requires further investigation^[52].

Platelets in IBD

Blood platelet levels may be considerably increased in active IBD both in CD and UC patients; this is a reaction to an intensified inflammatory process. Regardless of inflammatory process exacerbation, approximately 30%-50% of IBD patients develop spontaneous platelet

aggregation or platelet hypersensitivity to low concentrations of aggregating agents^[53]. The final stage of platelet hyperactivation has been found to be mediated by the CD40-CD40 ligand (CD40L) pathway. The surface CD40L is an activation marker that allows platelets to interact with a broad variety of immune and non-immune cells. It has been demonstrated that in IBD patients platelets overexpress CD40L protein up to four times more frequently than platelets from control subjects, and release more soluble CD40L (sCD40L) to the plasma, leading to a 15 fold increase in CD40L plasma levels. In general elevated levels of sCD40L are associated with an increased risk of TE development which is also true for patients with IBD^[53]. It has been observed that an increased platelet activity in IBD is also dependent on increased expression of surface activation markers, such as P-selectin and GP53 and on serum levels of platelet activation marker β -thromboglobulin^[34,54]. The higher platelet activity state mentioned above has been noted to be independent of clinical activity of the disease; the chronic disease process has been suggested to lead to increased platelet activity even in remission state^[34,54].

Finally, platelets are involved in chronic intestinal inflammation, what has been demonstrated in studies evaluating anus sections collected from IBD patients. Mucosal intravascular microthrombi have been shown both in CD and UC patients^[29,53]. In addition, the investigators have found that platelets of patients with IBD express high levels of surface CD40L, creating a physical and biological bridge that allows interaction with human intestinal microvascular endothelial cells causing their activation^[34,54], what leads to up-regulation of VCAM-1 and ICAM-1 by activated platelets through the CD40-dependent pathway and to increased production of IL-8 by endothelial cells, also through this pathway, and an increase in T cell adhesion to the endothelium^[34].

Autoantibodies

IBD patients have been observed to produce increased amounts of various antibodies; some of them are anti-coagulant antibodies and thus may increase the risk of thrombosis.

Antiphospholipid antibodies include anticardiolipin (aCL) antibodies and lupus anticoagulants (LAC). The antibodies may increase the risk of thrombosis through ac-

tivation of platelets and endothelial cells and by decreasing anticoagulant activity of proteins. In IBD patients as compared to the controls, the level of aCL antibodies is approximately 20%-30% higher, while the level of LAC antibodies is approximately 19% higher^[55,56].

In IBD, the prevalence of antibodies against β 2-glucoprotein I (β 2-GPI), the cofactor that mediates binding of aCL antibodies to cardiolipin, is higher than in the controls, with an average incidence of 9%^[56].

Antibodies against PS have been described in patients with IBD. The antibodies could reduce the natural anticoagulant potential^[48]. However, in up-to-date publications there is no good evidence that these antibodies play any role in thrombotic risk^[48].

Nevertheless, no significant differences have been demonstrated in the prevalence of the above antibodies in IBD patients with diagnosed TE as opposed to the IBD group without such complications^[48,56]. Further observations and studies are necessary to allow for a possible confirmation of the role of these antibodies in the development of TE.

Genetic factors

Genetic factors that have been implicated to play role in TE in IBD include FV Leiden (FVL, G1691A), the genetic variation of the prothrombin gene mutation (*G20210A*), methylenetetrahydrofolate reductase gene mutation (*MTHFR*, C677T), plasminogen activator inhibitor type 1 (*PAI-1*) gene mutation and FXIII (val34leu)^[20,34,57-59].

FVL is an arginine to glutamine missense mutation in the *FV* gene at position 506. FVL is the most frequent cause of inherited thrombophilia, it renders the activated FV form relatively resistant to degradation by activated protein C (APC), resulting in higher thrombin generation. The prevalence of FVL ranges from 20% to 30% in unselected patients with venous thrombosis^[20]. Most of studies have shown no difference in the prevalence of FVL between IBD patients and the healthy controls^[20,34], but in IBD patients with TE, the prevalence of FVL was significantly higher than in IBD patients without TE^[20]. Additionally, the prevalence of FVL in IBD patients with TE is comparable to its prevalence in non-IBD patients with TE^[58,60]. Although somewhat conflicting, these genetic studies suggest that FVL as a risk factor for TE in IBD patients matches that of the general population.

The *G20210A* gene mutation is the second most frequent genetic prothrombotic mutation after FVL. The prevalence of *G20210A* mutation is about 2% in healthy controls and 6.2% in patients with thrombosis^[60]. However, several studies have demonstrated the same prevalence of the gene mutation in IBD patients with and without TE^[60,61]. Some investigators have shown a close association between IBD and the presence of the *G20210A* mutation^[56,57].

Methylenetetrahydrofolate reductase (*MTHFR*) is a critical enzyme involved in the remethylation pathway of homocysteine metabolism. A common mutation (C677T)

has been identified in the *MTHFR* gene. The homozygous carries of this polymorphism are found in around 10% of the population and the polymorphism may be a cause of moderate hyperhomocysteinemia. Some studies have demonstrated a weak association between C677T homozygosity in the population and the risk of thrombosis^[62]. However, the study that associated the prevalence of C677T homozygosity between IBD thrombotic and IBD non-thrombotic subjects has shown no significant differences^[58,60].

The *FXIII* gene mutation (val34leu) is associated with a higher FXIII activation rate and leads to a 20%-40% risk reduction of venous thrombosis for homozygous carries, which are found in around 10% of the population^[63]. The FXIII mutation has been evaluated in IBD patients, but the available studies have not demonstrated any significant differences in the prevalence of this polymorphism in IBD patients with respect to the general population^[58,64].

PAI-1 gene mutation - the 4G/4G genotype - is associated with an overexpression of *PAI-1*, which may cause a decreased fibrinolysis. The 4G/4G genotype has been demonstrated to be a risk factor in myocardial infarction, arterial thrombosis and DVT^[20,65]. The available studies have demonstrated a higher prevalence of the genotype in IBD patients as compared to the general population^[65].

Investigations performed in IBD patients also assess other genetic hypercoagulability factors, such as deficiency of PC and PS, and ATIII mutation^[20].

Finally, in spite of numerous genetic studies performed in IBD, no unambiguous association has been demonstrated between genetic factors and causes of hypercoagulability in both CD and UC^[20,34].

Medications

Some medications used in the treatment of IBD patients may affect haemostatic system. 5-ASA used in a combination with oral anticoagulants might increase the risk of bleeding. Carty *et al.*^[66] observed that 5-ASA given orally or in vitro inhibits platelet activation.

It has been confirmed by many studies that glucocorticoids increase the risk of VTE^[67]. Johannesdottir *et al.*^[67] in a population-based case-controlled study observed a higher risk of VTE among present, new, continuing and recent glucocorticoids users but not among former ones. Glucocorticoids also inhibit oral anticoagulants.

Data regarding coagulation and use of anti TNF antagonists are conflicting. For instance, in a national prospective observational cohort study in Great Britain use of anti-TNF therapy was not associated with an increased risk of VTE in rheumatoid arthritis patients^[68]. However, the majority of publications confirm such a relationship^[69]. TEs have been noted in about 4.5% of patients treated with TNF antagonists. One of the possible explanations includes involvement of anti-drug antibodies that might be found in some patients. It has been speculated that antigen-Ab complexes could trigger thrombosis by activating either platelets or the comple-

ment system^[69]. Another hypothesis is based on predisposition of some patients to lupus-like reactions, including antiphospholipid syndrome^[69]. The inhibition of TNF leads to overproduction of interferon- α what might facilitate the development of lupus-like syndrome.

Concomitant use of thiopurines and anticoagulants may foster decrease in the effect of warfarin, what might be caused by reduced bioavailability, enhanced warfarin metabolism, or increased prothrombin activity^[70].

A meta-analysis of eight randomized-controlled trials performed in 2007 demonstrated that administration of heparin in patients with UC is safe, but does not give any benefit over conventional therapy^[71]. In 2010, a review of randomized trials confirmed no benefit of low molecular weight heparins (LMWH) administered subcutaneously over placebo for clinical remission induction in patients with UC. However, high dose LMWH administered *via* an extended colon-release tablet showed benefit over placebo for clinical remission and endoscopic improvement. There is no evidence to support the use of unfractionated heparin for the treatment of active UC^[72].

Thromboprophylaxis

There are no unambiguous indications to use thromboprophylaxis in patients with IBD^[73]. Many national guidelines support their use in this patient population^[74]. European Crohn's and Colitis Organisation (ECCO) suggests to consider prevention with both mechanical thromboprophylaxis and heparin in patients with UC at risk of TE and antithrombotic prophylaxis in all hospitalized patients with CD, especially in the event of prolonged immobilization^[75-78]. Evidence from randomized trials confirms that use of heparin and LMWH is generally safe in patients with IBD^[74]. Patients with IBD should be also informed about thrombotic risk factors such as oral contraceptive use and long-distance travel^[77,78].

CONCLUSION

Despite numerous studies, to date, the pathogenesis of IBD has not been unambiguously determined. The most commonly listed factors include genetic and immune abnormalities, although recently, discussions focus on the role of endothelial damage and coagulation disturbances as IBD-triggering factors. Persistent hypercoagulation may influence the clinical course of IBD and most likely is related to the interaction between chronic inflammatory process and coagulation cascade^[34]. Activation of coagulation acts as an element of the inflammatory response by directly mediating cytokine responses. Also hypofibrinolysis seems to be a typical feature of inflammation^[34]. That is why, the majority of TEs occur during the active phase of IBD^[77,78]. Acquired prothrombotic factors also play a crucial role in development of TE in IBD patients.

Further studies are necessary to assess the role of coagulation abnormalities in IBD etiology and to determine indications for thromboprophylactic treatment in patients

at high risk of developing TE.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Inflammatory pathways of importance for management of inflammatory bowel disease

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includes the TNF inhibitors (TNFi), designed to target and neutralise the effect of TNF- α . TNFi have shown to be efficient in treating moderate to severe CD and UC. However, convenient alternative therapeutics targeting other immune pathways are needed for patients with IBD refractory to conventional therapy including TNFi. Indeed, several therapeutics are currently under development, and have shown success in clinical trials. These include antibodies targeting and neutralising interleukin-12/23, small pharmacologic Janus kinase inhibitors designed to block intracellular signaling of several pro-inflammatory cytokines, antibodies targeting integrins, and small anti-adhesion molecules that block adhesion between leukocytes and the intestinal vascular endothelium, reducing their infiltration into the inflamed mucosa. In this review we have elucidated the major signaling pathways of clinical importance for IBD therapy and highlighted the new promising therapies available. As stated in this paper several new treatment options are under development for the treatment of CD and UC, however, no drug fits all patients. Hence, optimisations of treatment regimens are warranted for the benefit of the patients either through biomarker establishment or other rationales to maximise the effect of the broad range of mode-of-actions of the present and future drugs in IBD.

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Abstract

Inflammatory bowel disease (IBD) is a group of chronic disorders of the gastrointestinal tract comprising Crohn's disease (CD) and ulcerative colitis (UC). Their etiologies are unknown, but they are characterised by an imbalanced production of pro-inflammatory mediators, *e.g.*, tumor necrosis factor (TNF)- α , as well as increased recruitment of leukocytes to the site of inflammation. Advantages in understanding the role of the inflammatory pathways in IBD and an inadequate response to conventional therapy in a large portion of patients, has over the last two decades lead to new therapies which

Key words: Anti-tumor necrosis factor; Biologics; Crohn's disease; Pro-inflammatory cytokines; Signaling pathways; Treatment; Ulcerative colitis

Core tip: Crohn's disease and ulcerative colitis are the two prevailing forms of inflammatory bowel disease (IBD). Both diseases are associated with an increased expression of pro-inflammatory cytokines and immune cell infiltration of the inflamed tissue. Current treatment options with tumor necrosis factor- α inhibitors are discussed. Additionally, new therapeutic strategies

showing promising results, *e.g.*, small pharmacologic inhibitors aimed at inhibiting Janus kinase pathway and antibodies blocking recruitment of immune cells to the site of inflammation are also discussed. In this review we have elucidated the major signaling pathways of clinical importance for new therapeutic strategies of IBD.

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INTRODUCTION

The intestine is an important part of the immune system that recognises and reacts to environmental stimuli, including its luminal microbes. This interaction is highly regulated and allows commensal bacteria of the normal microbiome to exist without induction of mucosal inflammation, a mechanism known as intestinal homeostasis^[1]. Inflammatory bowel disease (IBD) is generally hypothesised to be a multifactorial condition where a combination of luminal, environmental, and genetic factors triggers an inappropriate mucosal immune response. As a consequence, an inappropriate and continuing inflammatory response to commensal microbes results in the spontaneous release of pro-inflammatory cytokines which challenge the mucosal homeostasis^[1]. These changes are to a some degree genetically determined and might lead to a dysfunction of the barrier integrity [mainly in ulcerative colitis (UC)], dysfunction in the sensing of microbes [mainly in Crohn's disease (CD)], and changes in the regulation of adaptive immune responses (in both UC and CD)^[2]. In IBD, this immune response does, however, not resolve, and the production of destructive inflammatory mediators continues. Inflammation in IBD is regulated by an increased secretion of a variety of pro-inflammatory mediators^[3], and it is noteworthy that concentrations of cytokine tumor necrosis factor (TNF)- α are elevated in the blood^[4], intestinal mucosa^[5,6] and stools^[7] of patients with IBD. In addition to TNF- α , the increased secretion of a variety of other pro-inflammatory mediators in stools and rectal dialysates from patients with IBD have been observed as well^[8-10].

An increased understanding of the involvement of inflammatory pathways combined with a lack of adequate response to conventional medical treatment has allowed biologics to emerge as an important modality in the treatment of patients with IBD. Several TNF inhibitors (TNFi) and other biologics as well as small molecules directed against pathways involving cytokines, adhesion molecules or other mediator systems involved in the pathogenesis of IBD have been developed and tested in clinical tri-

als^[3,11]. The aim of this review is from the present point of view to provide an overview of the pathways of inflammation which seems to be crucial for the management of IBD. Furthermore, a brief update on the latest clinical reports is given and promising new therapeutics are discussed.

REVIEW CRITERIA

The PubMed database was searched up to October 2013 using the following search terms: "TNF", "anti-TNF", "janus kinase", "Janus kinase/signal transducer and activator of transcription (JAK/STAT)", "tofacitinib", "ustekinumab", "IL-12/23", "natalizumab", "vedolizumab", "vercirnon", "integrins", "chemokines", "GSK-1605786A" individually or in combination with "IBD", "Crohn's disease", "ulcerative colitis", "inhibitors", "therapies", and "treatment". The search focused predominantly on full-text papers published in the English-language. Abstracts were included when critically relevant and when not already available as full-text articles. No publication date restrictions were applied. Subsequently, articles were selected for inclusion in this review on the basis of their relevance, and additional articles were obtained from their reference lists. Other sources of information were the websites of the European Medicines Agency, the United States Food and Drug Administration (FDA), and the Cochrane Library databases.

ANTI-TNF- α ANTIBODIES

A paradigm in management of IBD occurred in the late 90's when the pro-inflammatory cytokine, TNF- α , was identified as playing a pivotal role in the inflammatory cascade that orchestrate chronic intestinal inflammation^[12], and is today the best studied pro-inflammatory cytokine in IBD.

TNF- α is produced by mononuclear cells. Its synthesis is induced through activation of cellular receptors, *e.g.*, the Toll-like receptor 4 (TLR4). TLR4 is induced when derivatives from the cell wall of Gram negative bacteria, lipopolysaccharides, bind to TLR4 on the surface of mononuclear cells. Activation of TLR4 signaling induces activation of nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinases (MAPK), causing an increased cell proliferation and differentiation of macrophages as well as inducing expression of pro-inflammatory cytokines, *e.g.*, TNF- α , interleukin (IL)-6 and IL-12^[13,14]. The TNF- α protein exists in two forms: the precursor transmembrane form (tmTNF- α , 26 kDa) and the secreted soluble form (sTNF- α , 17 kDa). When synthesised, homotrimeric TNF- α translocates to the cell membrane where the TNF- α -converting enzyme releases the sTNF- α from tmTNF- α by proteolytic cleavage. The biological activity of TNF- α is mediated by its binding to TNF receptor type 1 (TNFR1) and type 2 (TNFR2)^[15]. After binding to the receptors, TNF- α initiates pro-inflammatory signaling by activation of the MAPKs

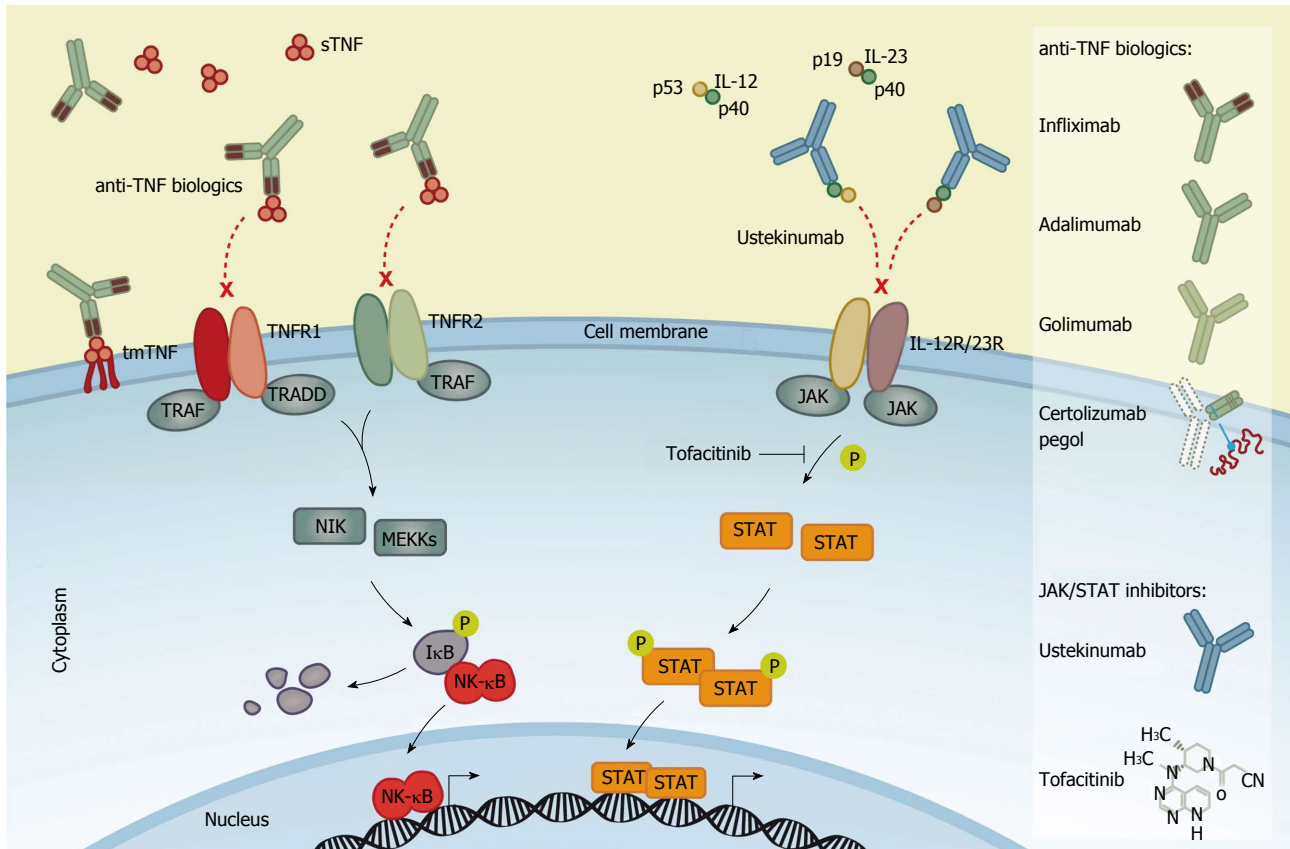


Figure 1 Mechanism of action of anti-tumor necrosis factor biologics and inhibitors of Janus kinase/signal transducer and activator of transcription signaling. Left panel: Anti-tumor necrosis factor (TNF) biologics bind to homotrimeric TNF- α [transmembrane (tmTNF- α) and/or soluble (sTNF- α)], thereby blocking the interaction between the cytokine and TNF- α receptor type 1 and 2 (TNFR1 and TNFR2) to neutralise TNF- α -mediated pro-inflammatory signaling. Infliximab is shown as a representative anti-TNF biologics to illustrate the mechanism of action of the four agents labeled for IBD: Infliximab (a chimeric antibody), adalimumab and golimumab (fully human antibodies), and certolizumab pegol (a pegylated Fab fragment of a humanised anti-TNF antibody). Right panel: The fully human antibody against interleukin (IL)-12/23, ustekinumab, binds to the common p40 subunit of IL-12 and IL-23 heterodimers and prevents the interaction of IL-12 and IL-23 with their cognate receptors, IL-12R and IL-23R, hence neutralising IL-12/23-mediated intracellular signaling. Tofacitinib is a small molecule inhibitor of Janus kinase (JAK) activity that prevents the JAK-dependent phosphorylation of signal transducer and activator of transcription (STAT) proteins and subsequently the STAT-induced transcription of pro-inflammatory target genes.

and NF- κ B pathways (Figure 1). Although, the active MAPKs and NF- κ B signaling pathways are important for homeostasis, they induce cell proliferation, differentiation and up-regulation of several pro-inflammatory cytokines, including TNF- α during inflammation^[16]. Additionally, TNF- α signaling induces caspase-8 activation and apoptosis of intestinal epithelial cells through TNFR1 signaling^[17], as well as inducing changes in the epithelial expression of tight junction proteins among patients with CD^[18]. Hence increased TNF- α expression might decrease the mucosal barrier function in patients with IBD exacerbating the inflammation.

Recent biologic therapies targeting TNF- α , with synthetic anti-TNF- α antibodies, have been shown to mitigate the inflammatory drive in chronic inflammatory conditions, including IBD. Initially, a chimeric (25% murine and 75% human sequence) IgG1 κ subclass antibody, infliximab, that specifically binds TNF- α was shown to be beneficial in CD^[19,20]. Attempts to reduce immunogenic responses induced by anti-chimeric antibodies included the removal of all murine sequences to create a fully human monoclonal antibody. This human IgG1 anti-TNF- α

monoclonal antibody, adalimumab, was also shown to be efficacious^[21,22], as was a pegylated humanised antibody fragment (binding TNF- α) lacking the IgG1 Fc portion and conjugated with a 40-kDa polyethylene glycol molecule, certolizumab pegol (Figure 1)^[23-25]. The observed efficacy in patients with CD provided a rationale for trials in patients with UC. Thus, in active stages of UC, an increased production of TNF- α by the mononuclear cells of the colonic lamina propria exists^[26], with high concentrations of TNF- α found in rectal dialysates and stools^[8]. Recent studies have shown another human IgG1 anti-TNF- α monoclonal antibody, golimumab, to be efficient in UC^[27]. However, the pharmacodynamics of TNFi seems to depend on the properties of the structure rather than simply the TNF- α -binding capacities. Eterncept, a non-antibody soluble recombinant TNF receptor (p75) Fc fusion protein, is not efficient for the treatment of intestinal inflammation^[28]. The reason for this difference remains unclear although several mechanisms have been proposed. Hence, the underlying modes of action of the various TNF inhibitors are much more complex than initially thought^[29]. The construct of the four TNFi's labeled

for IBD is shown in Figure 1.

Several placebo-controlled trials have demonstrated that infliximab^[20,30], adalimumab^[21,22,31], and certolizumab pegol^[24] are efficacious in moderate to severe CD, as both first- and second-line therapy in patients with inadequate responses to standard treatment^[32], including maintenance of response and remission^[25,30,33,34]. Not only do TNFi reduce signs and symptoms of disease, but they also allow tapering of glucocorticoids (steroid-free remission)^[21,30] and promote mucosal healing^[35]. The former is considered a clinically relevant benefit, and the latter suggests a positive effect on disease progression. Infliximab is also labeled for use in fistulising CD^[19,36].

In UC, randomised, controlled trials have shown infliximab^[37], adalimumab^[38,39], and golimumab^[27,40] to be effective in inducing and maintaining clinical remission (including steroid-free remission) in patients with moderate to severe disease activity who have failed conventional therapy with glucocorticoids and thiopurines, and in the ACT2 study, infliximab also was effective in outpatients refractory to 5-ASA^[37]. A *post hoc* analysis of the ACT1 and ACT2 studies showed that infliximab reduced the risk of colectomy after 1 year from 17% in the placebo group to 10% in the infliximab group (number needed to treat = 14)^[41]. Furthermore, infliximab^[37], adalimumab^[39], and golimumab^[27] additionally induced mucosal healing. Apart from use in patients with moderate chronic refractory UC, TNFi may be used as rescue therapy in hospitalised patients with severe UC. In a small placebo-controlled study, a single infusion of infliximab significantly reduced the number of colectomies among patients with an acute moderate to severe attack of UC^[42], and this was also observed in a subsequent open-label randomised, controlled trial with a high number of steroid-refractory acute severe UC patients, leading to the conclusion that the effect of infliximab did not differ from that of cyclosporine^[43].

The availability of TNFi has significantly altered the management of IBD in the last decade. The concomitant treatment with biologics and thiopurines proved in larger trials like the SONIC study to be superior for steroid-free clinical remission and absence of ulcerations (mucosal healing) at weeks 26 compared to monotherapy with either biologics or thiopurines in CD^[44]. The UC SUCCESS trial^[45] with a similar design and number of patients concluded the same, and the conclusion from both studies is that IBD patients in need of anti-TNF- α treatment should preferably receive combined treatment with a thiopurine. It should be emphasized that the use of potent immunomodulators (*i.e.*, thiopurines^[46]) in combination with TNFi is linked to an increased risk of hepatosplenic T-cell lymphoma^[47]. Even though data are confounded by the fact that most patients with IBD on TNFi therapy receives concomitant therapy with thiopurines, no casual associations between TNFi alone and the development of lymphoma have been identified^[11,47].

Although anti-TNF- α agents are the most effective anti-cytokine treatment in IBD a significant proportion

of patients does not respond to induction therapy (*i.e.*, “primary failures”) or loses the effect over time to become intolerant (*i.e.*, “secondary failures”) ^[48]. The development of antibodies to the available biological agents is not only responsible for secondary loss of effect, but it is also associated with a risk of infusion reactions, although concomitant immunomodulator therapy may reduce the magnitude of the immunogenic response^[48]. It has been shown that both adaption and switching from one TNFi to another can be efficacious to overcome resistance to anti-TNF- α treatment^[48]. Nonetheless, there clearly remains an unmet need for novel treatment options in IBD.

Extraintestinal manifestations occur frequently with a negative impact of the quality of patients' life^[49]. Some extraintestinal manifestations such as arthritis, erythema nodosum, pyroderma gangraenosum, iritis, and uveitis have a pathogenic TNF- α -dependent mechanism common with CD and UC, and TNFi might additionally be applied in such cases to dampen inflammation^[49].

JAK/STAT PATHWAY AND THERAPEUTIC ADVANCES IN IBD

Cytokines, which are small proteins produced by immune cells, facilitate communication between cells and have essential functions in cell development and differentiation. In addition to activate the NF- κ B and MAPK pathways^[16], a large number of mammalian cytokines exert their intracellular signaling by activating the JAK/STAT pathway^[50]. The JAK/STAT pathway is an evolutionary conserved signaling network involved in a wide range of cellular processes, including cell growth, survival, differentiation, proliferation, apoptosis, and inflammation^[51]. There are four members of JAKs - JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2) - that transduce signals through seven members of the STAT transcription factors - STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 - in diverse combinations^[50]. The diversity in cytokine responses is constituted by the combination of receptor binding to multiple JAKs and subsequent signaling through dimerised tyrosine-phosphorylated STAT variants. JAK1, JAK2, and TYK2 proteins can bind to various types of cytokine receptor families^[50] and are expressed ubiquitously, whereas JAK3 is specifically associated with the IL-2R family [also known as the common γ -chain (γ_c) family which includes receptors for interleukins such as IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, and IL-21] expressed only on hematopoietic cells^[52-54]. In addition to the γ_c family of receptors, JAKs can be activated by the common β -chain family (known as the IL-3R family which includes receptors for *e.g.*, IL-3 and IL-5), the receptors for IL-6, IL-11, IL-12, IL-23 and IL-27 cytokines which uses a glycoprotein-130 co-receptor (known as the IL-6R family), and the interferon (IFN)-R family [*i.e.*, receptors for IFNs (IFN- α , β , and γ), IL-10, IL-19, IL-20, IL-22, IL-25, and IL-26]^[50]. When STAT proteins become tyrosine-phosphorylated

by JAKs they form homo- or heterodimers which translocate from the cytoplasm into the nucleus to regulate gene expression of target genes (Figure 1).

Tofacitinib (JAK inhibitor)

There has been great interest in development of small molecule inhibitors of intracellular signaling pathways related to various conditions, including IBD. Several genetic knockout studies have demonstrated the importance and essential functions of JAKs in cytokine signaling (reviewed in details in references^[55-58]). Knockout of *Jak1* or *Jak2* genes are lethal in mice^[59,60], whereas dysfunction of *Jak3* or *Tyk2* in both mice and humans causes primary immunodeficiency^[61-64], underlying their importance for immune competence. Thus, the involvement of JAKs in a range of essential cytokine pathways has made JAK inhibitors a potential therapeutic target in IBD. Over the last two decades small-molecule JAK inhibitors have been synthesised and are currently under clinical investigation^[65]. Tofacitinib (formerly known as CP-690,550) was the first selective JAK inhibitor to be tested in human clinical trials. Tofacitinib inhibits all four JAKs, however, with functional specificity for JAK1 and JAK3 in cellular assays^[65,66]. Consequently, as a JAK1 and JAK3 inhibitor, tofacitinib effectively inhibits the signaling of the IL-2R family of cytokines^[50,65] and the receptor for IL-6 family of cytokines including IL-12 and IL-23^[53]. Tofacitinib also inhibits, albeit to a lesser extent, the IFN-R family^[67] as well as the IL-3 and IL-5 receptors. Thus, tofacitinib affects both the innate and adaptive immune responses by suppressing differentiation of Th1 and Th2 cells and affecting the pathogenic Th17 cytokine production^[65,68].

Tofacitinib is at present (September 2013) the only oral administered JAK inhibitor approved by FDA for use in therapy of adults with moderately to severely active rheumatoid arthritis (RA). However, there are investigations indicating that the drug can be effective in treatment of other chronic inflammatory indications such as UC. In a double-blind randomised controlled phase II trial in UC, patients treated with oral tofacitinib showed higher clinical response after 8 wk compared with placebo^[69]. The study comprised a total of 194 patients with moderate to severe UC receiving tofacitinib or placebo twice daily. Clinical response at 8 wk were found in 32%, 48%, 61%, and 78% of patients receiving twice daily tofacitinib at a dose of 0.5 mg ($P = 0.39$), 3 mg ($P = 0.55$), 10 mg ($P = 0.10$), and 15 mg ($P < 0.001$), respectively, as compared to 42% among patients receiving placebo^[69]. Similarly, clinical remission at 8 wk were associated with a dose-dependent improvement of 13% (0.5 mg, $P = 0.76$), 33% (3 mg, $P = 0.01$), 48% (10 mg, $P < 0.001$), and 41% (15 mg, $P < 0.001$) as compared with 10% of patients receiving placebo^[69]. Thus, tofacitinib seems effective and reasonably in patients with moderate to severe UC. In contrast, treatment of 139 randomised patients with moderate to severe CD with tofacitinib in a 4-wk phase II trial showed no clinical efficacy at doses of 1, 5, and 15 mg twice daily^[70]. The underlying difference between

the clinical efficacy of tofacitinib in UC and CD is unclear. With its oral route of administration, tofacitinib may offer a convenient alternative therapeutic option for UC patients who are refractory to conventional therapy such as anti-TNF- α therapy. However, larger long-term clinical studies with tofacitinib are required to report long-term safety as well as its therapeutic benefits in clinical use.

Ustekinumab (anti-IL-12/23 antibody)

One of the cytokine receptor families using the JAK/STAT signaling pathway is the IL-6 family of receptors. This receptor family includes receptors for IL-12 and IL-23 which are both heterodimeric cytokines consisting of two protein subunits, namely p35/p40 and p19/p40 subunits, respectively, hence sharing the p40 subunit^[71]. IL-12 binds to the IL-12R which is composed of IL-12R β 1 and IL-12R β 2 subunits, whereas IL-23 binds to the IL-23R complex, composed of IL-23R and IL-12R β 1^[72].

The IL-12R is primarily expressed by activated T-cells and natural killer (NK) cells, but has been found to be expressed on dendritic cells (DCs) and B-cells as well^[73,74]. IL-12 induces activated T-cells to differentiate into IFN- γ producing Th1 cells and it induces the NK cells to secrete IFN- γ and TNF- α ^[75,76]. The IL-23R is expressed on T-cells, but has also been found to be expressed by NK cells^[72,77]. IL-23 primarily induces proliferation and survival of Th17 cells. Th17 cells secrete high amounts of IL-17 and IL-17F together with TNF- α , IL-6, IL-21 and IL-22. These cytokines have been found to be implicated in chronic inflammation as well as autoimmune diseases^[78-81]. A study found IL-12 to be important for systemic activation of the immune system and IL-23 to drive local intestinal inflammation. Additionally, IL-23 was found to be involved in intestinal inflammation, because it was possible to reverse active colitis by administration of anti-IL-23, which might inhibit the pathogenic Th17 response, in a murine model^[82]. In support of this, an increased expression of IL-23 and IL-17 has been found in the lamina propria of CD patients^[83-85]. Likewise, overexpression of IL-12 and IL-23 has been found in the inflamed mucosa of CD patients^[86-88]. Exactly how these two inflammatory cytokines contributes to the pathogenesis of CD has yet to be determined.

Ustekinumab is a human IgG1 monoclonal antibody designed to bind to the p40 subunit common to both IL-12 and IL-23, thereby neutralising their activity (Figure 1). The binding of ustekinumab to free IL-12 and IL-23 cytokines block their interaction with IL-12R β 1^[89] expressed on T-cells, NK cells, macrophages, DCs, and B-cells. Ustekinumab was approved for treatment of psoriasis in 2009^[90,91]. In recent years, testing of anti-IL-12/23 treatment in patients with CD has been performed. In a double-blind, cross-over phase II trial with moderate to severe CD, patients were divided into two populations; the first population (I) enrolled 104 patients who had previously received conventional or bio-

logic therapy, and the second population (II) comprised patients whom were non-responders to infliximab^[92]. In population I, 49% of ustekinumab treated patients were in clinical response after 8 wk (the primary end points) as compared to 40% of patients with placebo ($P = 0.34$). However, ustekinumab treatment was efficient in patients who had previously received infliximab (population II). Overall clinical response following 8 wk of ustekinumab treatment in population II was significantly greater than the group receiving placebo ($P < 0.05$). Hence, in the fall 2012, Sandborn *et al.*^[93] revealed a placebo-controlled phase II trial including 526 patients with moderate to severe CD who had all failed anti-TNF- α therapy. After 6 wk of randomised induction therapy (1, 3, or 6 mg/kg of body weight) or placebo, 145 patients had a clinical response to ustekinumab, as compared to placebo (1 mg, $P = 0.02$; 3 mg, $P = 0.06$; 6 mg, $P = 0.005$). At week 6, these 145 patients were enrolled in a second randomisation to receive either placebo or 90 mg ustekinumab at weeks 8 and 16 as a maintenance therapy. While the clinical remission rates in the induction therapy did not differ significantly from placebo at week 6, the maintenance therapy with ustekinumab every 8 wk resulted in significantly higher clinical remission at week 22 (42%), as compared with placebo (27%, $P = 0.03$). Therefore, ustekinumab may induce clinical response in moderate to severe CD among patients who are refractory to infliximab.

In order to demonstrate evidence of definitive effectiveness and safety of ustekinumab, phase III trials are needed to test the long-term effects of ustekinumab as well as possible side effects. Three phase III trials are currently under way. They include an induction trial in patients with moderate to severe CD who are either naïve to anti-TNF- α treatment or have previously failed anti-TNF- α treatment as well as a maintenance trial in patients who respond to induction treatment with ustekinumab^[94].

TRAFFICKING OF THE IMMUNE CELLS TO THE INTESTINE

Following pathogen invasion of the intestinal mucosa and initiation of an immunogenic response, a priming of effector cells occurs in the Peyer's patches or in the mesenteric lymph nodes (MLNs). After the T-cell priming and release into the circulation, specific adhesion molecules are required for the cells to home to the site of inflammation. Important adhesion molecules in the homing to the intestines are the chemokine receptor 9 (CCR9) and the integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$, which are all induced on intestinal mucosal T-cells following activation and imprinting of gut-homing specificity^[95,96].

The homing of immune cells to the intestine during inflammation has become a major target in the treatment of IBD. In order to reduce leukocyte infiltration and continuation of inflammation, a number of molecules have been targeted by antibodies and small molecules to block these adhesion molecules^[3].

Natalizumab and vedolizumab (Integrin blocking antibodies)

In mammals, 18 α -integrin and 8 β -integrin subtypes have been discovered which are able to assemble into at least 24 individual integrin heterodimers^[97]. Of importance for IBD are two integrin heterodimers, namely $\alpha 4\beta 1$ and $\alpha 4\beta 7$, which at present have been utilised for treatment. Following inflammation of the small or large intestine a significant increase is observed in the endothelial expression of both ligands of these integrins. These are the mucosal addressin cell adhesion molecule-1 (MAdCAM-1)^[98] and vascular cell adhesion molecule-1 (VCAM-1)^[99], respectively. The primed leukocytes in the circulation are in this way recruited to the inflamed intestine in UC and CD, where they are able to extravasate the endothelium to reach the site of inflammation.

The first drug to be approved for targeting cell adhesion molecules in IBD was natalizumab, a recombinant humanised monoclonal IgG4k antibody (Figure 2). It has in some countries been approved for CD^[100], for both induction and maintenance indications. Natalizumab is an antibody targeting the $\alpha 4$ integrin subunit, hence targeting both the $\alpha 4\beta 1$ and the $\alpha 4\beta 7$ integrin heterodimers^[101,102] (Figure 2). The ligands of these two integrins are located on the endothelium in the intestinal lamina propria, but additionally the $\alpha 4\beta 1$ integrin is also involved in targeting of leukocytes to the central nervous system (CNS) through interaction with endothelial VCAM-1^[96,103] (Figure 2). Natalizumab has for this reason been approved for multiple sclerosis (MS) due to its ability to limit neuronal inflammation and to reduce the risk of progressing disability^[100,104]. This effect of natalizumab on blocking leukocyte adhesion to the CNS is considered to be a side effect as it thereby inhibits immune surveillance of the CNS. Three cases of progressive multifocal leukoencephalopathy (PML) in patients on natalizumab have been reported among two patients with MS receiving concomitant IFN- β -1 α ^[103,105], and one patient with CD on monotherapy^[106]. Following blockade of the leukocyte recruitment and patrolling of the CNS, these patients experienced a reactivation or infection of the opportunistic JC virus leading to PML. Hence, natalizumab is today used as a second-line therapy, and patients might be screened for JC virus infection prior to treatment and are not allowed to be on concomitant immunomodulators^[100].

Natalizumab is today the only available biological therapy for CD not targeting TNF- α and have shown convincing results. In the final randomised placebo-controlled phase III study, 509 patients with moderate to severe CD experienced clinical response rates by week 4 of 51% and 37% ($P = 0.001$) for natalizumab and placebo treatment, respectively, falling to 48% and 32% at week 8 ($P < 0.001$) (300 mg natalizumab or placebo at weeks 0, 4, and 8). Sustained remission occurred in 26% vs 16% of the patients following week 12 ($P = 0.002$)^[102]. A meta-analysis performed on 5 randomised controlled trials of natalizumab has furthermore shown natalizumab to be

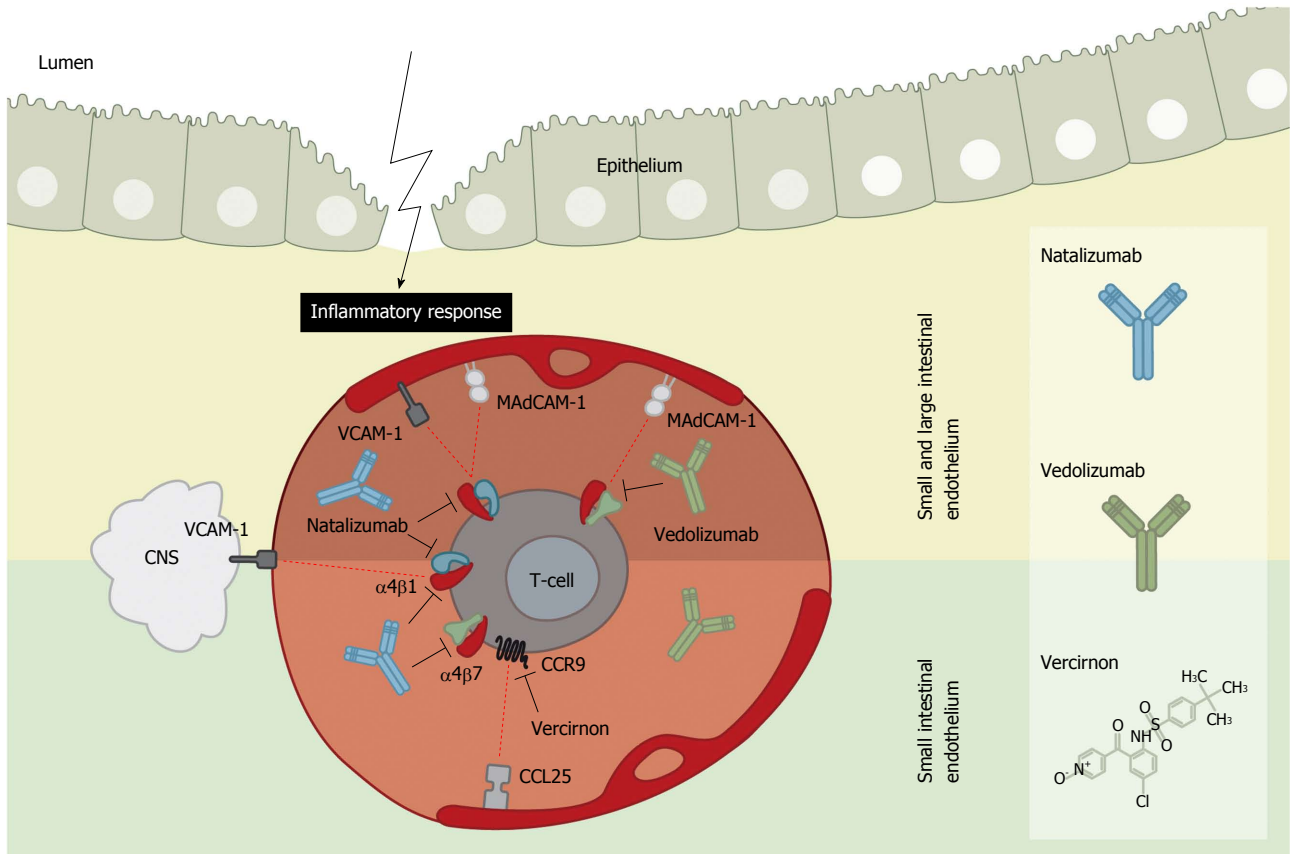


Figure 2 Integrin and chemokine inhibitors in intestinal endothelium. Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and vascular adhesion molecule-1 (VCAM-1) adhesion molecules become up-regulated on the endothelium during intestinal inflammation. In the small and large intestine (upper panel) the adhesion molecule MAdCAM-1 functions as docking site for integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$ expressed by T-cells. Contrary, the adhesion molecule VCAM-1 also acts as docking molecule for $\alpha 4\beta 1$. Addition of the inhibitory $\alpha 4\beta 1$ antibody, natalizumab, thus blocks adhesion of T-cells to the entire intestinal mucosa. As VCAM-1 is additionally expressed within the central nervous system (CNS), natalizumab also blocks T-cell extravasation and hence limits immune surveillance of the CNS which might lead to progressive multifocal leukoencephalopathy. Using the $\alpha 4\beta 7$ inhibitory antibody, vedolizumab, only the adhesion of T-cells to the intestine specific MAdCAM-1 is blocked, and thus does not limit immune surveillance of the CNS while dampening the inflammatory response. In the endothelium of the small intestine (lower panel) the chemokine ligand 25 (CCL25) adhesion molecule is predominantly expressed during inflammation. It acts as a ligand for the chemokine receptor 9 (CCR9) on T-cells. The inhibitor vercirnon blocks the CCL25-CCR9 chemotaxis thus inhibiting T-cells adhesion to the small intestinal mucosa. Vercirnon was recently withdrawn following a phase III trial.

superior to placebo^[107].

Due to the risk of PML following natalizumab treatment, more specific antibodies are, however, warranted. Vedolizumab is a humanised monoclonal IgG1 antibody specific for the $\alpha 4\beta 7$ integrin, hence blocking leukocyte adhesion to MAdCAM-1 expressed solely by the gut endothelium^[103] (Figure 2). On the basis of the phase III study series for vedolizumab (GEMINI Studies™) Takeda filed a Biologics License Application (BLA) to the FDA for use in both UC and CD in the early summer of 2013^[108]. Years back, randomised placebo-controlled phase II trials showed clinical effect of a monoclonal antibody similar to vedolizumab on both CD and UC^[109,110]. Results from the GEMINI series (with GEMINI III and GEMINI LTS still ongoing) showed that patients with UC, responding to vedolizumab induction therapy, receiving 300 mg vedolizumab every 4 or 8 wk for 52 wk had remission rates of 49% ($P < 0.001$) and 42% ($P < 0.001$), respectively, compared to remission rates of 16% in the placebo group. Following a 6 wk induction period

the clinical remission rates were 47% *vs* 26%, respectively for vedolizumab over placebo ($P < 0.001$)^[111]. For CD, a clinical remission rate of 15% compared to 7% ($P = 0.02$) was observed for vedolizumab (300 mg) compared to placebo following 6 wk induction treatment. Prolonged treatment of responders with vedolizumab every 4 or 8 wk over the following 52 wk showed clinical remission rates of 36% ($P = 0.004$) and 39% ($P < 0.001$), respectively, compared to 22% in the placebo group^[112].

There are no concerns for PML in relation to vedolizumab as it does not seem to influence the immune surveillance of the CNS in contrast to natalizumab^[103,113]. There are high expectations to vedolizumab which is the only gut selective drug for treatment of IBD, and it may potentially be used as either a secondary intervention following anti-TNF- α failure or as a first-line treatment.

Integrins are important in the process of cellular adhesion underlying the mechanism of action for the integrin blocking antibodies. Integrins do, however, also transmit cellular signaling. Evidence suggests that the

interaction of the $\alpha 4$ integrins ($\alpha 4\beta 1$ and $\alpha 4\beta 7$) with their ligands lead to the binding of its cytosolic domain to paxillin, an intracellular signaling adaptor protein^[114]. Hence, paxillin is a part of the focal adhesion complex and gets phosphorylated by focal adhesion kinase and Src kinase as well as other kinases including c-Jun N-terminal kinases (JNK) and p38 MAPK^[115]. Mutations of the cytoplasmic tail of $\alpha 4$ integrin disrupts the paxillin binding site and lead to a reduced recruitment of mononuclear leukocytes to the site of thioglycollate-induced peritonitis^[116], although the integrin binding to its ligands was unaffected. This evidence suggests that in particular the $\alpha 4$ integrin signaling might influence the leukocyte homing to the inflamed section of the gastrointestinal tract or may even play part in the full T-cell activation process by blockade of nuclear factor of activated T-cells (NFAT) signaling, despite the major effect as being an adhesion molecule itself^[115]. These findings may provide insight to the discipline of regulating the inflammatory response; however, this specific area needs further investigation.

Vercirnon (chemokine receptor inhibitor)

In addition to integrins, CCR9 (a G-protein coupled receptor) also play a role in immune cell recruitment to the intestine by exerting a chemotatic response towards chemokine ligand 25 (CCL25)^[117]. CCR9 is preferentially expressed on thymocytes^[118] as well as on intraepithelial and lamina propria lymphocytes of the small intestine^[119]. CCL25 is considered a potential intestine-specific homing ligand expressed both within the crypts of the intestinal epithelium^[120], and on the vascular endothelium within the human small intestine^[121,122]. CCL25 is also expressed in the colon but in much lower levels as compared to the small intestine^[123].

The involvement of CCR9 in lymphocyte trafficking to the small intestine was initially suggested by the fact that CCR9 is selectively expressed on gut-homing T-cells^[124]. The CCR9⁺ T cells have also been found to be elevated in the peripheral blood of CD patients, but not in patients with CD solely confined to the colon^[125]. In this context, inhibition of CCL25/CCR9 chemotaxis has been suggested to be an attractive target for CD of the small intestine. Oral administration of vercirnon (GSK-1605786A), a selective small-molecule antagonist of CCR9 that inhibits CCR9- and CCL25-dependent chemotaxis (Figure 2) in an experimental mouse model demonstrated a clear therapeutic benefit by leading to significantly reduced inflammation in these mice^[126,127]. In addition, *in vitro* experiments with human peripheral blood mononuclear cells showed that vercirnon is a selective and potent antagonist of human CCR9^[126] and inhibits both functional variants of CCR9 (CCR9A and CCR9B)^[126,128]. Based on these findings, several clinical double-blind, placebo-controlled phase II trials (reviewed in reference^[127]) have been conducted for shorter and longer induction periods with vercirnon in patients with moderate to severe CD. The clinical remission rate following 36 wk of vercirnon treatment with 250 mg twice

daily was 47% as compared to 31% in the placebo group. In addition, the levels of C-reactive protein were normalised in 19% of subjects receiving vercirnon compared with only 9% of individuals given placebo^[127]. In a recent study the expression of CCR9 was also observed on activated regulatory T-cells and is important for trafficking these cells to the inflamed site of the intestine^[129] suggesting that inhibition of CCR9- and CCL25-dependent chemotaxis may result in a dysfunctional immune response and consequently exacerbation of CD. Moreover, a newly published clinical double-blind, placebo-controlled multicenter phase II trial (Prospective Randomized Oral-Therapy Evaluation in Crohn's disease Trial-1) for assessing the efficacy and safety of vercirnon in moderate to severe CD patients showed that vercirnon was well tolerated with no elevated risk of disease exacerbation compared to placebo^[130]. However, in September 2013, GlaxoSmithKline (GSK) decided to terminate all clinical trials with vercirnon as it failed in the first phase III study, SHIELD-1, both the primary endpoint of improved clinical response as well as the key secondary endpoint of clinical remission. SHIELD-1 was a randomised, double-blind and placebo-controlled study to evaluate the efficacy and safety of two doses (500 mg once daily and 500 mg twice daily) of vercirnon compared to placebo over 12 wk in 608 adults with moderate to severe CD. The study showed no efficacy with either dose in inducing a clinical response or remission. Moreover, increased rates of adverse events of vercirnon, including gastrointestinal- and cardiac disorders as compared to placebo were reported.

CONCLUSION

In recent years progress in basic and translational research has led to better understanding of the role of inflammatory mediators in the pathogenesis of IBD. It is believed that an altered balance between regulatory and inflammatory cytokines contributes to perpetuate the mucosal inflammation in both CD and UC. Since there is evidence that the tissue damaging immune response is driven by multiple cytokine driven inflammatory pathways, it is logical to hypothesise that simultaneously targeting two or more of these signals could be more advantageous than inhibiting just selective single pathways. Various approaches for inhibiting such pathways have been developed and are ready to go into the clinic. However, in designing clinical interventions around these new drugs it should be taken into consideration that inhibition of inflammatory cytokines could be associated with severe side-effects, as these molecules are also involved in the regulation of physiological processes and immune responses against infections and neoplasias. Another promising therapeutic strategy is to restore counter regulatory mechanisms which are defective in IBD. Since it is conceivable that no drug fits all patients, further experiments will be necessary to identify biomarkers that predict responsiveness to the anti-cytokine based therapy as

well as to ascertain which biological therapy will be most effective for the individual patient (*i.e.*, tailored therapy).

Analysis of immuno-inflammatory pathways in the gut of patients with CD and UC have shown that tissue damage is driven by complex and dynamic cross-talk between immune and non-immune cells, and that cytokines are key mediators of this interplay^[1,131]. Considerable efforts have furthermore been used on the regulation of the inflammatory cellular invasion into the lamina propria to break the continuous pathogenic cytokine signaling. It has been demonstrated that some cytokine mediated counter-regulatory anti-inflammatory pathways are defect in IBD raising the possibility that restoring these anti-inflammatory signals may be a therapeutic strategy. These advances together with several experimental models of intestinal inflammation have facilitated the development of components and biologics that neutralise cytokines. Some of these drugs have already been tested in patients with IBD and others are ready to move into clinical trials. However, given the plethora of immunological manipulations that can prevent colitis in experimental models, the recent failures of anti-IFN- γ , and anti-IL-17 antibodies as well as the antagonist of CCR9 in the clinical setting, and the fact that only 66% and 50% of patients with CD and UC, respectively, respond to anti-TNF- α therapy, it now becomes extremely difficult to decide which other cytokines/chemokines should be targeted, and if they will ever beat TNF α ^[132].

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Surgery for luminal Crohn's disease

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Abstract

Many patients with Crohn's disease (CD) require surgery. Indications for surgery include failure of medical treatment, bowel obstruction, fistula or abscess formation. The most common surgical procedure is resection. In jejunoileal CD, strictureplasty is an accepted surgical technique that relieves the obstructive symptoms, while preserving intestinal length and avoiding the development of short bowel syndrome. However, the role of strictureplasty in duodenal and colonic diseases remains controversial. In extensive colitis, after total colectomy with ileorectal anastomosis (IRA), the recurrence rates and functional outcomes are reasonable. For patients with extensive colitis and rectal involvement, total colectomy and end-ileostomy is safe and effective; however, a few patients can have subsequent IRA, and half of the patients will require proctectomy later. Proctocolectomy is associated with a high incidence of delayed perineal wound healing, but it carries a low recurrence rate. Patients undergoing proctocolectomy with ileal pouch-anal anastomosis had poor functional outcomes and high failure rates. Laparoscopic surgery has been introduced as a minimal invasive procedure. Patients who undergo laparoscopic surgery have a more rapid recovery of bowel

function and a shorter hospital stay. The morbidity also is lower, and the rate of disease recurrence is similar compared with open procedures.

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Key words: Crohn's disease; Laparoscopic surgery; Resection; Strictureplasty; Surgery

Core tip: Strictureplasty is now an accepted procedure in the management of jejunoileal Crohn's disease (CD). However, the place for strictureplasty is less well defined in duodenal and colonic diseases. For patients with extensive colonic CD, the surgical choices include total colectomy with either an ileorectal anastomosis or end-ileostomy, or a total proctocolectomy with permanent end-ileostomy. Patients with CD undergoing ileal pouch-anal anastomosis are associated with poor functional outcomes and high failure rates. Laparoscopic surgery is safe and feasible. Patients who undergo laparoscopic surgery have a more rapid recovery of bowel function and a shorter hospital stay.

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INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disease of unknown cause that can involve any portion of the digestive tract. Inflammation can extend entirely through the intestinal wall, and most patients with CD will eventually develop stricturing or perforating complications^[1]. Accordingly, the majority of patients require surgery as part of the therapeutic management of their disease. Probability of surgical resection within 15 years after diagnosis was 70%^[2]. CD may be helped by surgery, but it

cannot be cured by surgery. The primary goals of surgery are to alleviate serious complications, achieve the best possible quality of life (QOL), and conserve as much bowel as possible.

INDICATIONS FOR SURGERY

Indications for surgery include failure of medical treatment, bowel obstruction, fistula or abscess formation, or combination of these indications. The majority of patients are treated with elective surgery. However, patients with intestinal perforation, peritonitis, excessive bleeding or toxic megacolon require urgent surgery. Improved medical treatment may lead to higher rates of elective operations^[3]. However, prolonged conservative treatment may also increase the number of serious complications before surgery. Therefore, a multidisciplinary approach with early involvement of the surgeon is important to avoid any delay in indication to surgery.

SURGICAL OPTIONS

In 1987 Alexander-Williams and Haynes^[4] formulated the 5 “Golden Rules” of surgical management of CD (Table 1). Surgical treatments for CD include bypass, resection, and strictureplasty. The aim of surgery for CD has shifted from radical operation, achieving inflammation-free margins of resection, to minimal surgery, intended to remove just grossly inflamed tissue or performing strictureplasty. The surgeon must assess which procedure is most suitable at each area of the disease.

Bypass

There are two types of bypass operations: exclusion bypass and simple (continuity) bypass. For certain types of ileocecal CD with associated with serious abscess or phlegmon densely adherent to the retroperitoneum, the proximal cut end of the transected ileum is anastomosed to the transverse colon in an end-to-side fashion with or without construction of a mucus fistula of the distal cut end of the ileum (exclusion bypass) or an ileotransverse colonic anastomosis is made in a side-to-side fashion (simple bypass). In the study by Homan and Dineen^[5], CD involving the ileum and cecum was treated with resection in 115 patients, exclusion bypass in 25, and simple bypass in 21. Overall recurrence rates were 25% for resection, 63% for exclusion bypass, and 75% for simple bypass. This difference was accounted for by early recurrence or by persistent disease in the two bypass groups: 21% for exclusion bypass and 45% for simple bypass as compared to 3% for resection. These results indicate that the recurrence rate following resection is significantly lower than bypass, and continuing disease in the bypassed loop accounts for a high percentage of reoperations in the bypass groups. Resection is the surgical treatment of choice for ileocecal CD. Furthermore, there is the risk of malignancy in the bypassed segment^[6]. At present, bypass surgery is rarely performed except for gastroduodenal

CD. The surgical management of gastroduodenal CD is discussed later.

Resection

Resection margin: The most commonly performed operation for patients undergoing surgical management of their CD is resection of the diseased segment. In small bowel disease, the involved segment is characterized by thickening of the mesenteric margin of the bowel, fat wrapping on the sides of the intestinal wall^[7]. The non-diseased bowel can be distinguished from diseased bowel by palpating the mesenteric border of the bowel wall. Several studies^[8,9] suggested that radical resection, the wide resection of normal bowel uninvolved microscopically, was associated with a lower recurrence rate as compared with non-radical resection. Other studies^[10,11] have shown no difference in the recurrence rates between patients treated with radical resection and non-radical resection. In a randomized controlled trial (RCT)^[12], 152 patients undergoing ileocolonic resection for CD were randomly assigned to two groups in which the proximal line of resection was 2 cm (limited resection) or 12 cm (extended resection) from the macroscopically involved area. With a median follow-up time of 56 mo, disease recurred in 25% of patients in the limited resection group and 18% of patients in the extended resection group (not significant). This study indicate that recurrence rate is unaffected by the width of the margin of resection from macroscopically involved bowel segment. Radical resection is not recommended because it does not reduce recurrence rates.

Anastomotic technique: Stapled functional end-to-end anastomosis has become a popular procedure in colorectal surgery. Its potential benefits include a wide anastomotic lumen, minimal contamination, and a quick method. Fecal stasis and subsequent bacterial overgrowth are implicated in anastomotic recurrence in CD. A wider anastomosis is less likely to cause a functional obstruction, and may be associated with a lower risk of recurrence. Several studies^[13-15] reported that the stapled functional end-to-end anastomosis was associated with fewer postoperative complications and recurrences as compared with sutured end-to-end anastomosis (conventional anastomosis). In a meta-analysis, the outcomes were compared between end-to-end anastomosis and other anastomotic configurations after bowel resection for CD^[16]. Eight studies reported on 661 patients who underwent 712 anastomoses, of which 383 (53.8%) were sutured end-to-end anastomosis and 329 (46.2%) were other anastomotic configurations (259 stapled side-to-side, 59 end-to-side or side-to-end, 11 stapled circular end-to-end). End-to-end anastomosis was associated with increased anastomotic leak rates. Side-to-side anastomosis was associated with fewer anastomotic leaks and overall postoperative complications, and a shorter hospital stay. There was no significant difference between the groups in perianastomotic recurrence and reopera-

Table 1 Five "Golden Rules" of surgical management of Crohn's disease^[4]

Crohn's disease is a panintestinal disease, with intermittent activity and the potential of focal exacerbations throughout the patient's life
It is impossible to cure Crohn's disease by excision. The surgeon is required only to treat the complications
The essence of surgical treatment is to make the operation as safe as possible. If the operation becomes safe and patients survive, they will inevitably have recurrences and so repeated operations may be required. Therefore, it is important to conserve as much gut as possible
All diseased bowels need not be excised, only that part with complications
If only stenotic complications are being treated, perhaps the stenosis can be simply widened by strictureplasty or dilatation

Table 2 Contraindications to strictureplasty

Excessive tension due to rigid and thickened bowel segments
Perforation of the intestine
Fistula or abscess formation at the intended strictureplasty site
Hemorrhagic strictures
Multiple strictures within a short segment
Malnutrition or hypoalbuminemia (< 2.0 g/dL)
Suspicion of cancer at the intended strictureplasty site

tion needed because of perianastomotic recurrence. Five of the 8 studies included in this meta-analysis were non-randomized retrospective trials. In an RCT^[17], 139 patients who underwent an ileocolonic resection were randomized to side-to-side anastomosis (STSA group) or end-to-end anastomosis (ETEA group). There were no significant differences in overall complication rates (24% in the ETEA group *vs* 20% in the STSA group), anastomotic leak rates (7% in the ETEA group *vs* 7% in the STSA group) and reoperative rates for complications (7% in the ETEA *vs* 7% in the STSA group). After a mean follow-up of 11.9 mo, the symptomatic recurrence rate was 21.9% in the ETEA group, compared with 22.7% in the STSA group (not significant). The endoscopic recurrence rate was 42.5% in the ETEA group, compared with 37.9% in the STSA group. Based on these results^[16,17], CD recurrence after bowel resection is not affected by anastomotic type.

Kono *et al*^[18] has recently introduced a new antimesenteric functional end-to-end handsewn anastomosis (Kono-S anastomosis) designed to minimize anastomotic restenosis in patients with CD. In their retrospective study, 69 patients with Kono-S anastomosis (group S) were compared with 73 historical patients with conventional anastomosis (group C) from 1993 to 2003. The frequency of endoscopic recurrence at anastomosis was comparable between the two groups (83% at 1 year and 100% at 5 years in group S *vs* 79% at 1 year and 100% at 5 years in group C). However, the median endoscopic recurrence score at the anastomosis was significantly lower in group S than in group C (2.6 *vs* 3.4). At 5 years after operation, surgical recurrence rate at the anastomosis was significantly lower in group S than in group C. The Kono-S anastomosis appears to be effective in preventing anastomotic surgical recurrence in CD. However, to rigorously evaluate the efficacy of the Kono-S anastomosis, large RCTs are necessary.

Strictureplasty

Indications: One of the risks of repeated or extensive resections for small bowel CD is the development of short bowel syndrome. The rationale for strictureplasty is to avoid resection and preserve intestinal length and

function of the small bowel^[19,20]. Strictureplasty is ideal for diffuse involvement of the small bowel with multiple strictures, and strictures in patients who have undergone extensive resections or multiple resections of the small bowel. Strictures of duodenum, and ileocolonic or ileorectal anastomosis can be treated with strictureplasty. In contrast, contraindications to strictureplasty are presented in Table 2.

At laparotomy, strictures are identified by careful palpation of the bowel. The Heineke-Mikulicz strictureplasty is used for short strictures of up to 10 cm in length. In contrast, the Finney strictureplasty is used for longer strictures of up to 25 cm. Heineke-Mikulicz strictureplasty rarely develops metabolic sequelae, which may suggest that absorptive capacity is preserved. Whether the bowel is similarly functionally preserved after Finney strictureplasty is open to question, and some would argue that the bacterial overgrowth and blind-loop syndrome may result from this type of strictureplasty. Very long strictures (> 30 cm) and multiple strictures within a short segment have traditionally been treated with resection. Several authors have introduced new types of strictureplasty for these conditions^[21-28]. We will discuss this in more detail towards the end of this section.

Outcomes: In a meta-analysis^[19], a total of 1112 patients who underwent 3259 strictureplasties (Heineke-Mikulicz 81%, Finney 10%, side-to-side isoperistaltic 5%, others 4%) were identified. The sites of strictureplasty were jejunum and/or ileum (94%), previous ileocolonic or ileorectal anastomosis (IRA) (4%), duodenum (1%), and colon (1%). After jejunoileal strictureplasty, including ileocolonic anastomotic strictureplasty, septic complications (anastomotic leak, enteric fistula, intra-abdominal abscess) occurred in 4% of patients. Poor nutritional status, anemia, peritoneal contamination due to intraabdominal sepsis, older age and emergency operation were risk factors for the complications after strictureplasty. In contrast, steroid use, synchronous bowel resection, and number, site or type of strictureplasties were not significant factors. Overall surgical recurrence was 23% (95%CI: 17%-30%). The 5-year recurrence rate after strictureplasty was 28%. In 90% of patients, recurrence occurred at non-strictureplasty sites, and the site-specific recurrence rate was 3%. Younger age was a risk factor for recurrence after strictureplasty. Few patients developed a short bowel syndrome. These results confirmed that strictureplasty may have largely replaced resection and minimized the

risk of the short bowel syndrome.

Strictureplasty vs resection: There was concern about a potential increase in septic complications and disease recurrence with strictureplasty, as this procedure preserves diseased bowel and as the suture line is fashioned through macroscopic disease. It is difficult to compare the outcomes of strictureplasty and resection, since resection is principally used for severely affected segments which are not suitable for strictureplasty. There have been no RCTs comparing the outcomes between strictureplasty and resection. In a recent meta-analysis^[29], seven studies comprising 688 patients (strictureplasty 45%, resection with/without strictureplasty 55%) were analyzed. Patients undergoing strictureplasty alone had a lower risk of developing postoperative complications than those who underwent resection (OR = 0.60, 95%CI: 0.31-1.16) although this was not statistically significant. Surgical recurrence after strictureplasty was more likely than after resection (OR = 1.36, 95%CI: 0.96-1.93). Patients who had a resection showed a significantly longer recurrence-free survival than those undergoing strictureplasty alone (HR = 1.08, 95%CI: 1.02-1.15). These results suggest that patients with small bowel CD undergoing strictureplasty alone may have fewer postoperative complications than those undergoing a concomitant bowel resection. However, surgical recurrence maybe higher following strictureplasty alone than with a concomitant small bowel resection. RCTs are necessary to rigorously compare the outcomes between strictureplasty and resection.

New types of strictureplasties: Multiple strictureplasties in a short segment may result in a bulky and unyielding segment of intestine, which leads to considerable tension on each suture line. In patients with long and rigid strictures, the Finney strictureplasty may not be technically feasible because the intestinal wall lacks the pliability to fold onto itself. Michelassi^[22] initially proposed a new technique, the side-to-side isoperistaltic strictureplasty in the management of long strictures or multiple strictures in a short segment. Other authors have also introduced their new types of strictureplasties^[24-28]. A prospective study^[30] reported on the results of 184 patients who underwent the side-to-side isoperistaltic strictureplasty in six centers. The average length of diseased bowel selected for the strictureplasty varied among centers, from 20.8 to 64.3 cm. The complication rate after surgery varied from 5.7% to 20.8%. The most commonly encountered complication was gastrointestinal hemorrhage (2%), followed by suture line dehiscence (1%), and bowel obstruction (1%). Forty-one of the 184 patients required surgery for recurrent disease, with an average time to recurrence of 35 mo. The cumulative reoperation-free rate at 5 years after surgery was 77% across all centers. This study suggests that the side-to-side isoperistaltic strictureplasty carries a low mortality and morbidity rate, with acceptable recurrence rates.

A recent systematic review compared short-term and

long-term results between conventional (Heineke-Mikulicz and Finney) and non-conventional (modified Finney, combined Heineke-Mikulicz and Finney, modified Heineke-Mikulicz, Michelassi, and modifications of it and others) strictureplasties^[31]. One thousand one hundred fifty-seven patients underwent conventional strictureplasties with an early complication rate of 15% *vs* 459 patients underwent non-conventional strictureplasties with an early complication rate of 8%. A late complication rate was 29% for the conventional strictureplasty group *vs* 17% for the non-conventional strictureplasty group. The early and late complication rates are comparable between the non-conventional and conventional strictureplasty procedures. However, bowel function is preserved after long strictureplasty is open to discussion because motility disturbances may occur after this type of strictureplasty. Michelassi *et al*^[23] found that in their enteroclysis study, flow of contrast was unimpeded, and no stasis or retention of contrast occurred in the strictureplasty site. Further investigation is necessary to evaluate the efficacy of the new types of strictureplasties.

SURGERY FOR SPECIFIC CONDITIONS

Duodenal CD

Gastroduodenal CD is an uncommon condition, occurring in about 1%-5% of all CD cases^[32,33]. The most common site of gastroduodenal CD was the duodenal bulb, and gastroduodenal CD was almost invariably present in association with disease elsewhere in the intestinal tract^[32,33]. Stricture was the most common pathology of gastroduodenal CD^[32,33]. Presence of symptoms of obstruction needs medical therapy with steroids and immunomodulatory drugs including infliximab. When these medications do not alleviate the symptoms, balloon dilation or surgery is the option to consider. In patients with gastroduodenal CD, the most common indication for surgery was obstruction.

Several authors reported the outcomes of surgery for gastroduodenal CD. In the Birmingham study^[34], 26 patients with obstructive duodenal CD were treated with strictureplasty ($n = 13$) or bypass surgery ($n = 13$). The median duration of follow-up was 159 mo. In the strictureplasty group, 2 patients developed an anastomotic breakdown leading to an enterocutaneous fistula. Obstructive symptoms were not improved in four patients, of whom 3 patients were managed with conservative treatments and the other patient required a gastrojejunostomy. In the long-term, 6 patients developed restructre at the previous strictureplasty site, of whom 5 required repeat strictureplasty and the other patient underwent duodenojejunostomy. Overall, 9 patients after strictureplasty required further surgical treatment due to 3 early postoperative complications (anastomotic leaks 2 and persistent obstructive symptoms 1) and 6 restructres at the previous strictureplasty site. In the bypass group, 3 patients had persistent obstructive symptoms and 1 developed intra-abdominal sepsis in the early postopera-

tive period. All settled on conservative treatments. In the long-term, 6 patients required further surgery for stomal ulceration ($n = 2$) and anastomotic obstruction ($n = 4$). In the Cleveland Clinic study^[35], 34 patients with duodenal CD were treated with strictureplasty ($n = 13$) or bypass ($n = 21$). Strictureplasty was associated with 2 postoperative complications, and 1 patient required reoperation for recurrence at a mean follow-up of 3.6 years. Bypass was associated with 2 postoperative complications, and 1 patient required reoperation for recurrence at a mean follow-up of 8 years. In the management of duodenal CD, strictureplasty has the advantage of avoiding a blind loop syndrome or stomal ulceration. In the Birmingham study, strictureplasty has no obvious advantages over bypass surgery. In contrast, the Cleveland Clinic study shows that strictureplasty is safe and effective, and has potential physiologic advantage over bypass. Thus, the results between these studies are very different probably because of differences in disease aggressiveness and follow-up duration. Most recently, the outcomes of surgery for duodenal CD in 10 patients were reported^[36]. Eight patients were treated with strictureplasty, and 2 patients were treated with resection: 1 with a gastroduodenal resection, and 1 with a duodenojejunal resection and an end to side duodenojejunal anastomosis. No recurrence of duodenal CD was observed in the 2 patients treated with resection, while 2 of the 8 patients treated with strictureplasty developed recurrence. The authors suggest that duodenal strictureplasty is indicated when less than 2 strictures are present in the second or third duodenal portion. In cases with multiple strictures localized in the first or the distal duodenal portion, resection is preferable. They also suggest that an accurate complete mobilization of the duodenum is an important step of the procedure, as it allows sutures without tension. In the previous studies, the number of patients is too small to conclude on the efficacy of duodenal strictureplasty. Further studies in larger cohorts of patients are necessary.

Diffuse jejunoileal CD

Diffuse jejunoileal disease (multiple segments of disease in the proximal ileum and jejunum) is relatively uncommon, the incidence being 3%-10% of entire CD population^[37]. Diffuse jejunoileal disease is one of the most difficult areas to treat in CD. Resectional surgery may lead to short bowel syndrome. A retrospective study^[38] reported on the long-term outcomes of surgical treatment for diffuse jejunoileal CD. Forty-six patients required surgery for diffuse jejunoileal CD. During an initial operation, strictureplasty was used on 63 strictures in 18 patients (39%). After a median follow-up of 15 years, 39 patients (85%) required 113 reoperations for jejunoileal recurrence. During 75 of the 113 reoperations (66%), strictureplasty was used on 315 strictures. Only two patients developed short bowel syndrome and required home parenteral nutrition. At the end of the study, 4 patients were symptomatic and required medical treatment. All other patients were asymptomatic and required neither medical

treatment nor nutritional support. Most patients with diffuse jejunoileal CD can be restored to good health with minimal symptoms by surgical treatment that includes strictureplasty.

The Cleveland Clinic reported their experience of strictureplasty for diffuse jejunoileal CD^[39]. One hundred twenty-three patients underwent strictureplasty for diffuse jejunoileitis. Total number of strictureplasties performed was 701 (median, 5 per patient). Seventy percent of patients underwent a synchronous bowel resection. The overall morbidity rate was 20%, with septic complications occurring in 6%. The surgical recurrence rate was 29% with a median follow-up period of 6.7 years. The recurrence rate in diffuse jejunoileitis patients did not differ from that seen in 219 patients with limited small bowel CD undergoing strictureplasty.

Extensive colorectal CD

For patients with extensive colonic CD, the surgical choices include total colectomy with either an IRA or end-ileostomy, or a total proctocolectomy with permanent end-ileostomy. Several authors^[40-52] reported on the outcomes of surgery for extensive Crohn's colitis. A retrospective study^[47] reviewed 144 patients who underwent a total colectomy for Crohn's colitis. IRA was performed in 118 patients, while 26 never had an IRA after colectomy because of severe anorectal lesions. The probability of clinical recurrence after IRA was 58% and 83% at 5 and 10 years respectively. The probability of rectal preservation at 5 and 10 years was 86% and 86% after IRA. Patients with extraintestinal manifestation had a higher risk of recurrence and of rectal preservation failure. In the literature review, the rate of functional IRA after total colectomy for extensive Crohn's colitis at 10 years was approximately 70% (Table 3). One study^[45] reviewed 69 patients who underwent a total colectomy and end-ileostomy with an oversewn rectal stump for extensive Crohn's colitis. Only 5 patients (7%) underwent IRA, of whom 2 required proctectomy later. Overall, 37 patients (54%) required proctectomy, with a median duration of 2 years. Sixteen patients (23%) developed small bowel recurrence requiring surgery, with a median duration of 6.8 years. In the literature review, the rate of secondary proctectomy for defunctioned rectum after a total colectomy with end-ileostomy is presented in Table 4. Half of the patients will subsequently require proctectomy after a total colectomy with end-ileostomy. In a retrospective study^[52], 103 patients who underwent single-stage proctocolectomy for extensive colorectal CD were reviewed. The commonest postoperative complication was delayed perineal wound healing (35%), followed by intra-abdominal sepsis (17%) and stomal complications (15%). In 23 patients the perineal wound healed between 3 and 6 mo after proctocolectomy, whereas in 13 patients the wound remained unhealed for more than 6 mo. There were 2 hospital deaths (2%) caused by sepsis. The 5-, 10-, and 15-year cumulative reoperation rates for small bowel recurrence were 13%, 17%, and 25%, respectively, after a

Table 3 Rate of functional ileorectal anastomosis after a total colectomy for extensive Crohn's colitis at 10 years

Ref.	n	Functioning ileorectal anastomosis
Buchmann <i>et al</i> ^[40]	105	70%
Ambrose <i>et al</i> ^[41]	63	71%
Longo <i>et al</i> ^[42]	118	48%
Chevallier <i>et al</i> ^[43]	83	63%
Pastore <i>et al</i> ^[44]	42	65%
Yamamoto <i>et al</i> ^[45]	65	78%
Bernell <i>et al</i> ^[46]	106	76%
Cattan <i>et al</i> ^[47]	118	86%

median follow-up of 18.6 years. Thus, proctocolectomy for CD is associated with a high incidence of complications, particularly delayed perineal wound healing. However, proctocolectomy carries a low recurrence rate in the long-term. A recent literature review confirms an approximately 30% risk of recurrence of CD after an end ileostomy^[53]. A penetrating phenotype and preexisting ileal disease are risk factors for disease recurrence. A thorough evaluation of the stoma/peristomal area and evaluation of the small bowel by ileoscopy and small bowel imaging are required to assess the extent of disease and extraluminal complications such as stomal retraction and fistulas that require further surgical intervention. While postoperative medical treatment with immunosuppression or biological therapy is often employed, these therapies are unproven to prevent postoperative recurrence in the setting of a stoma.

In the management of extensive colitis, after a total colectomy with IRA, the recurrence rates and functional outcomes are reasonable if the rectum is not severely affected and sphincter function is not compromised. For patients with rectal involvement, total colectomy and end-ileostomy is safe and effective; however, a few patients can have subsequent IRA, and half of the patients will require proctectomy later. Proctocolectomy is associated with a high incidence of complications, particularly delayed perineal wound healing, but it carries a low recurrence rate. Algorithm for surgical treatment of extensive colitis is presented in Figure 1.

Ileal pouch surgery for CD

Ileal pouch-anal anastomosis (IPAA) is the surgical treatment of choice for most patients with ulcerative colitis (UC)^[54,55]. In contrast, CD is considered a contraindication to IPAA. The unexpected diagnosis of CD after IPAA operation is a relatively frequent occurrence. Several authors^[56-58] reported the outcomes of IPAA for patients with CD. Hartley *et al*^[56] reviewed 60 patients who underwent IPAA for UC subsequently had that diagnosis revised to CD. With a median follow-up of 46 mo, pouch loss rate was 12%. Median daily bowel movement in those with IPAA *in situ* was seven (range 3-20), with 50% of patients rarely or never experiencing urgency and 59% reporting perfect or near perfect continence. In 1985, Panis *et al*^[57] decided to investigate an alternative to total proctocolectomy with definitive end-ileostomy by a

Table 4 Rate of secondary proctectomy for defunctioned rectum after a total colectomy with end-ileostomy for extensive Crohn's colitis

Ref.	n	Follow-up (yr)	Secondary proctectomy
Harling <i>et al</i> ^[48]	59	7.7	50%
Guillem <i>et al</i> ^[49]	47	6	51%
Sher <i>et al</i> ^[50]	25	6	40%
Yamamoto <i>et al</i> ^[51]	65	10	54%

prospective study of IPAA for selected patients with CD. Between 1985 and 1992, 31 patients with CD, but with no evidence of anoperineal or small bowel disease, were recruited to their study. All patients underwent IPAA. The short-term and long-term functional results of this procedure were compared with those of 71 UC patients who also underwent IPAA during the same period in their unit. With a mean follow-up of 59 mo, no significant differences were observed between patients with CD and UC in the postoperative complication rate. Of the 31 patients with CD, 6 (19%) experienced specific complications nine mo to 6 years after surgery: 3 had pouch-perineal fistulas, which required pouch excision in 2 cases, 1 had a pouch-vaginal fistula that was treated by gracilis muscle interposition, and 1 had an extrasphincteric abscess, which was treated surgically. Two patients (6%), 1 of whom was treated for an extrasphincteric abscess, experienced CD recurrence on the reservoir, and were treated successfully with azathioprine. At 5-year follow-up, there were no significant differences between patients with CD and UC in stool frequency (5.0 *vs* 4.7 per day), continence, gas/stool discrimination, leak or need for protective pads, and sexual activity. Their results show that in selected cases of CD without anoperineal or small bowel manifestations, IPAA can be recommended as an alternative to proctocolectomy with definitive end-ileostomy. In 2001, the same research group reported 10-year results of IPAA in selected patients with colorectal CD^[58]. Forty-one patients underwent IPAA for colorectal CD between 1985 and 1998. None had past or present history of anal manifestations or evidence of small bowel involvement. Follow-up was 113 mo, 20 patients having been followed for more than 10 years. Eleven (27%) patients experienced CD-related complications, 47 mo after IPAA: 2 had persistent anal ulcerations with pouchitis and granulomas on pouch biopsy and were treated medically, 2 experienced extrasphincteric abscesses and 7 presented pouch-perineal fistulas which were treated surgically. Among them, 3 patients with persistent perineal fistula despite surgery required definitive end-ileostomy. Of the 20 patients followed for more than 10 years, 7 (35%) experienced CD-related complications which required pouch excision in 2 (10%). These good long-term results justify for surgeons to propose IPAA in selected patients with colorectal CD.

Melton *et al*^[59] reviewed 204 CD patients (108 female, median age 33 years, and median follow-up 7.4 years) with primary IPAA. CD diagnosis was before IPAA (in-

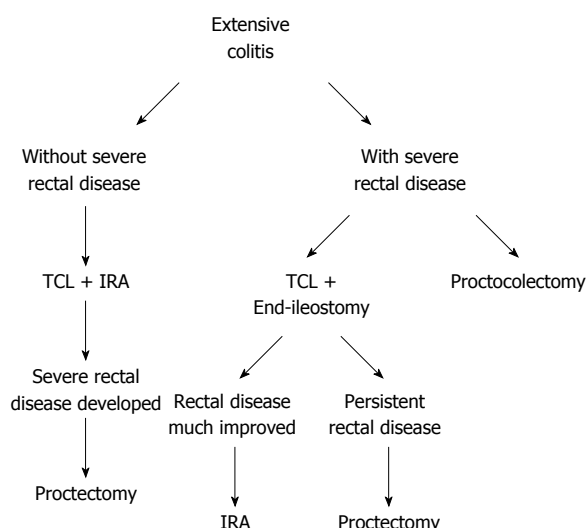


Figure 1 Algorithm for surgical treatment of extensive colitis. TCL: Total colectomy; IRA: ileorectal anastomosis.

tentional) in 20 (10%), from postoperative histopathology (incidental) in 97 (47%) or made in a delayed fashion at median 36 mo after IPAA in 87(43%). Overall 10-year pouch retention was 71%. On multivariate analysis, pouch loss was associated with delayed diagnosis (HR = 2.6, 95%CI: 1.1-6.5), pouch-vaginal fistula (HR = 2.8, 95%CI: 1.3-6.4), and pelvic sepsis (HR = 9.7, 95%CI: 3.4-27.3). Patients with retained IPAA at follow-up had near-perfect/perfect continence (72%), rare/no urgency (68%) with median daily bowel movements 7 (range 2-20). Median overall QOL, quality of health, level of energy, and happiness with surgery were 9, 9, 8, and 10 of 10, respectively. For CD patients with IPAA, when the diagnosis is established preoperatively or immediately following surgery, pouch loss rates are low and functional results are favorable. Outcomes in patients with delayed diagnosis are worse but half retain their pouch at 10 years with good functional outcomes. Most recently, Le *et al*^[60] reported the surgical outcomes of IPAA when used intentionally for well-defined CD. Seventeen patients with preoperative CD were identified, whereas the preoperative diagnosis was UC in 261 patients. Seven of the 17 patients (41%) in the preoperative CD group developed postoperative CD (recurrence in the afferent limb 3, pouch fistulizing disease 4) *vs* 27 of the 261 patients (11%) in the UC group (afferent limb inflammation 23, perianal disease 4) ($P = 0.002$). Of the seven CD patients with recurrent inflammation, three patients were maintained on immunosuppressive therapy and another three patients were controlled with antibiotics alone. Only one patient (6%) of the preoperative CD patient cohort with severe pouch inflammation and perianal disease required pouch excision and permanent ileostomy after failing aggressive medical therapy. The incidence of pouch failure was not statistically significant between patient groups.

A meta-analysis compared postoperative complications and functional outcomes after IPAA between

patients with CD and those with non-CD diagnoses^[61]. Ten clinical trials comprising 3103 patients (CD 225, UC 2711, indeterminate colitis 167) were included. Patients with CD developed more anastomotic strictures than non-CD diagnoses (OR = 2.12, $P = 0.05$) and experienced pouch failure more frequently than patients with UC (CD *vs* UC: 32% *vs* 4.8%, $P < 0.001$; CD *vs* indeterminate colitis: 38% *vs* 5%, $P < 0.001$). Urgency was more common in CD compared with non-CD: 19% *vs* 11% ($P = 0.02$). Incontinence occurred more frequently in CD compared with non-CD patients: 19% *vs* 10% (OR = 2.4, $P = 0.01$). Twenty-four-hour stool frequency did not differ significantly between CD, UC, or indeterminate colitis. Patients with isolated colonic CD were not significantly at increased risk of postoperative complications or pouch failure ($P = 0.06$). If surgeons were to undertake IPAA in the presence of CD, then the patient would require careful counseling to outline the risk of poorer functional outcomes and higher failure compared with non-CD patients. If patients are prepared to accept these risks and meet the selection criteria, such as isolated colonic CD without any evidence of terminal ileal or perianal involvement, there may be a role for IPAA in this select group of patients.

LAPAROSCOPIC SURGERY

Laparoscopic surgery is “minimally invasive” procedure commonly used to treat diseases of the gastrointestinal tract. Unlike traditional gastrointestinal surgery where a long incision down the center of the abdomen is required, laparoscopic surgery requires only small incisions in the abdomen. As a result, patients undergoing the procedure may experience less pain and scarring after surgery and a more rapid recovery. Several clinical studies have reported the outcomes of laparoscopic surgery for CD. These studies have reported conflicting results. Most of the studies have been limited by a small sample size and a short follow-up duration.

Several meta-analyses on efficacy of laparoscopic surgery for CD were conducted^[62,63]. Rosman *et al*^[62] found that laparoscopic surgery required more operative time than open surgery (26.8 min; 95%CI: 6.4-47.2), but resulted in a shorter duration of ileus and a decreased hospital stay (-2.62 d; 95%CI: -3.62--1.62). Laparoscopic surgery also was associated with a decreased rate for postoperative bowel obstruction and surgical recurrences. Tan and Tjandra^[63] found that the rate of conversion from laparoscopic to open surgery was 11.2%. Laparoscopic procedures took longer to perform compared with open procedures, with a weighted mean difference of 25.54 min ($P = 0.03$). Patients who underwent laparoscopic surgery had a more rapid recovery of bowel function, with a weighted mean difference of 0.75 d ($P = 0.02$) and were able to tolerate oral intake earlier, with a weighted mean difference of 1.43 d ($P = 0.0008$). The duration of hospitalization was shorter, with a weighted mean difference of 1.82 d ($P = 0.02$). Morbidity was lower for

laparoscopic procedures compared with open procedures (OR = 0.57, 95%CI: 0.37-0.87, $P = 0.01$). The rate of disease recurrence was similar for both laparoscopic and open surgery. From these results in the 2 meta-analyses, although laparoscopic procedures take longer to perform, patients who undergo laparoscopic surgery have a more rapid recovery of bowel function and a shorter hospital stay. The morbidity also is lower, and the rate of disease recurrence is similar compared with open procedures. Thus, laparoscopic surgery for CD is safe and feasible.

Long-term results of laparoscopic surgery *vs* conventional surgery for CD were evaluated in 2 RCTs^[64,65]. In the first RCT^[64], recurrence and complication rates were compared between laparoscopic (LC) and open ileocollectomy (OC) for ileocolonic CD. Mean follow-up for 56 patients (27 LC *vs* 29 OC) was 10.5 years and comparable between LC and OC (10.0 *vs* 11.0, respectively; $P = 0.64$). One patient died 8 years after OC of causes unrelated to CD. Eight patients for each group underwent initial reoperative (LC 26% *vs* OC 28%, $P = 0.89$). One patient underwent incisional hernia repair after LC (4%) *vs* 4 patients (14%) after OC ($P = 0.61$). Two patients in the LC group underwent adhesiolysis *vs* none after OC ($P = 0.23$). Incidences of anorectal disease, anorectal surgery, endoscopic or radiologic recurrence, and medication use were also similar between LC and OC. OC patients requiring operation during follow-up were significantly more likely than LC to require multiple operations ($P = 0.006$). This study confirms that LC is at least comparable to OC in the treatment of ileocolonic CD. In the second RCT^[65], 60 patients who underwent ileocolonic resection between 1999 and 2003 were studied. Five patients were lost to follow-up. Median follow-up was 6.7 years. Sixteen of 29 and 16 of 26 patients remained relapse free after ileocolonic resection in the laparoscopic and open groups respectively (risk difference 6, 95%CI: -20-32). Resection of recurrent CD was necessary in 2 of 29 *vs* 3 of 26 patients (risk difference 5, 95%CI: -11-20). Overall reoperation rates for recurrent CD, incisional hernia and adhesion-related problems were 2 of 29 *vs* 6 of 26 (risk difference 16, 95%CI: -3-35). QOL was similar, whereas body image and cosmesis scores were significantly higher after laparoscopy ($P = 0.029$ and $P < 0.001$, respectively). This study confirms that laparoscopically assisted ileocolonic resection results in better body image and cosmesis, whereas open surgery is more likely to produce incisional hernia and obstruction. These 2 RCTs indicate that laparoscopic surgery for small bowel CD is as safe as the open operation. There is no significant difference in the perioperative outcomes and the long term reoperation rates for disease-related or non-disease related complications of CD^[66].

PREOPERATIVE CONDITIONS AND RISK OF POSTOPERATIVE COMPLICATIONS

Nutritional status and septic conditions

Currently, the use of biological agents constitutes one

way to diminish local, and alleviate mucosal inflammation, thereby allowing surgery to be performed at complicated disease sites. However, evidence to date would suggest that there has been little change in the natural history of the disease and hence surgical therapies. Surgery provides good long-term disease control in many patients, and that delay in operating may result in more advanced disease and hence more postoperative complications^[67]. Yamamoto *et al*^[67] reviewed 343 patients who underwent 1008 intestinal anastomoses during 566 operations for primary or recurrent CD between 1980 and 1997. Intra-abdominal septic complications, defined as anastomotic leak, intra-abdominal abscess, or enterocutaneous fistula, developed after 76 operations (13%). Intra-abdominal septic complications were significantly associated with preoperative low albumin level (< 30 g/L), preoperative steroids use, abscess at the time of laparotomy, and fistula at the time of laparotomy. The intra-abdominal septic complication rate was 50% (8/16 operations) in patients with all of these 4 risk factors, 29% (10/35 operations) in patients with 3 risk factors, 14% (14/98 operations) in patients with 2 risk factors, 16% (33/209 operations) in patients with 1 risk factor, and 5% (11/208 operations) in patients with none of these risk factors ($P < 0.0001$). The following factors did not affect the incidence of septic complications; age, duration of symptoms, number of previous bowel resections, site of disease, type of operation (resection, strictureplasty, or bypass), covering stoma, and number, site, or method (sutured or stapled) of anastomoses. Preoperative low albumin level, steroid use, and the presence of abscess or fistula at the time of laparotomy significantly increased the risk of septic complications after surgery in CD. A surgical decision about avoiding an anastomosis must take into account many issues, especially age, degree of malnutrition, severity of coexisting sepsis and the dose of steroids or immunosuppressants. However, we must conclude from our data that an anastomosis is much more likely to break down if three or four of the four risk factors are present in a particular patient. It is not clear that preoperative nutritional intervention can reduce the risk of postoperative complications. Preoperative management of septic conditions may be associated with a lower incidence of complications after surgery. However, there has been no striking evidence that management of malnutrition and sepsis before surgery improves the surgical outcomes in CD. Delay in operation may be associated with an increased risk of serious complications. Further studies are necessary on these practical issues.

Impact of biologic therapy on postoperative complications

The immunosuppressive effects of preoperative anti-tumor necrosis factor (TNF)- α therapy may increase the risk for postoperative complications among CD patients undergoing abdominal surgery. A number of meta-analyses^[68-70] were conducted to compare the rates of postoperative complications among CD patients treated

with anti-TNF- α therapy *vs* alternative therapies. A total of eight studies including 1,641 patients were included in the meta-analysis by Kopylov *et al*^[68]. Preoperative infliximab therapy in CD patients undergoing abdominal surgery was associated with a trend toward an increased rate of total complications (OR = 1.72, 95%CI: 0.93-3.19). Anti-TNF- α treatments were associated with a modestly increased risk of infectious complications (OR = 1.50, 95%CI: 1.08-2.08), mostly remote from the surgical site (OR = 2.07, 95%CI: 1.30-3.30) and with a trend toward a higher rate of noninfectious complications (OR = 2.00, 95%CI: 0.89-4.46). Preoperative infliximab treatment was associated with an increased risk of postoperative infectious complications, mostly nonlocal. A trend toward an increased risk of noninfectious and overall complications was also observed. In the meta-analysis by Billioud *et al*^[69], the prevalence of infectious postoperative complications was increased in CD patients who underwent preoperative anti-TNF- α therapy (OR = 1.45, 95%CI: 1.03-2.05). In the meta-analysis by Rosenfeld *et al*^[70], data were extracted from 6 studies including 1159 patients among whom 413 complications were identified. The most common complications were wound infections, anastomotic leak and sepsis. There was no significant difference in the major complication rate (OR = 1.59, 95%CI: 0.89-2.86), minor complication rate (OR = 1.80, 95%CI: 0.87-3.71), reoperation rate (OR = 1.33, 95%CI: 0.55-3.20) or 30 day mortality rate (OR = 3.74, 95%CI: 0.56-25.16) between the infliximab and control groups. Thus, the impact of anti-TNF- α therapy on postoperative complications remains unclear.

POSTOPERATIVE MANAGEMENT FOR THE PREVENTION OF RECURRENCE

Risk factors for postoperative recurrence

In the surgical management of CD, postoperative recurrence is common, and many patients require repeat operation for recurrence. The reoperation rates for recurrence have been reported to be 10%-30% at 5 years, 20%-40% at 10 years and 40%-60% at 20 years after surgery^[71]. The reoperation rate tends to steadily increase with time, reaching approximately 50% at 20 years after surgery. The most significant factor affecting postoperative CD recurrence was found to be smoking^[71,72]. Smokers had an increased risk of recurrence compared to non-smokers. Similarly, perforating CD appeared to be associated with a higher recurrence rate compared with non-perforating CD^[71,72].

Monitoring for postoperative recurrence

Rutgeerts *et al*^[73] reported that recurrent lesions were observed endoscopically in the neo-terminal ileum (the proximal site of the ileocolonic anastomosis) within 1 year of resection in 73% of patients, although only 20% of the patients had symptoms. Three years after surgery, the endoscopic recurrence rate increased to 85% and symptomatic recurrence occurred in 34%. Patients with

severe endoscopic lesions within 1 year after resection developed early clinical recurrence. In contrast, patients with no or mild endoscopic lesions had a low frequency of subsequent clinical recurrence. The severity of the endoscopic inflammation in the neo-terminal ileum during the first year after resection was found to be a reliable predictive risk factor for future clinical recurrence. Ileocolonoscopy is the gold standard in the diagnosis of postoperative recurrence by defining the presence and severity of morphologic recurrence and predicting the clinical course. Ileocolonoscopy is recommended within the first year after surgery where treatment decisions may be affected.

Prophylactic medications

Prophylactic treatment is recommended after small intestinal resection^[74,75]. High dose mesalazine is an option for patients with an isolated ileal resection^[74,75]. Thiopurines are more effective than mesalazine or imidazole antibiotics alone for preventing both clinical and endoscopic recurrence^[74,75]. In patients with a risk factor for early postoperative recurrence the drug of choice is azathioprine/mercaptopurine^[74,75]. Imidazole antibiotics have been shown to be effective after ileocolonic resection but are less well tolerated. Probiotics have failed to show efficacy in postoperative CD recurrence, but should merit further investigations^[72,75]. There has been no RCT evaluating the efficacy of enteral nutrition in the prevention of postoperative CD recurrence^[72,75]. Several RCTs^[76-78] investigated the efficacy of biologic agents for the prevention of recurrence after resection for CD. Regueiro *et al*^[76] randomly assigned 24 patients who had undergone ileocolonic resection to receive intravenous infliximab (5 mg/kg), administered within 4 wk of surgery and continued for 1 year, or placebo. The primary end point was the proportion of patients with endoscopic recurrence at 1 year. Secondary end points were clinical recurrence and remission and histologic recurrence. The rate of endoscopic recurrence at 1 year was significantly lower in the infliximab group (1/11 patients; 9.1%) compared with the placebo group (11/13 patients; 84.6%). There was a nonsignificant higher proportion of patients in clinical remission in the infliximab group (8/10 patients; 80.0%) compared with the placebo group (7/13 patients; 53.8%). The histologic recurrence rate at 1 year was significantly lower in the infliximab group (3/11 patients; 27.3%) compared with the placebo group (11/13 patients; 84.6%). Administration of infliximab after intestinal resection was effective at preventing endoscopic and histologic recurrence of CD. In the study by Yoshida *et al*^[77], 31 patients who had ileocolonic resection within the past 4 wk were randomly assigned to scheduled infliximab at 5 mg/kg intravenously every 8 wk for 36 mo ($n = 15$) or without infliximab (control, $n = 16$). At 12 and 36 mo, 100% and 93.3% of patients in the infliximab group were in remission, respectively *vs* 68.8% and 56.3% in the control arm ($P < 0.03$). Further, the infliximab group achieved higher endoscopic remission at 12 mo (78.6%

vs 18.8%, $P = 0.004$). Savarino *et al*^[78] randomly assigned 51 patients who had undergone ileocolonic resection to receive after 2 wk from surgery adalimumab at the dose of 160/80/40 mg every 2 wk, azathioprine at 2 mg/kg per day, or mesalamine at 3 g/d, and they were followed up for 2 years. The primary end point was the proportion of patients with endoscopic and clinical recurrence. The rate of endoscopic recurrence was significantly lower in adalimumab (6.3%) compared with the azathioprine (64.7%; OR = 0.036, 95%CI: 0.004-0.347) and mesalamine groups (83.3%; OR = 0.013, 95%CI: 0.001-0.143). There was a significantly lower proportion of patients in clinical recurrence in the adalimumab group (12.5%) compared with the AZA (64.7%; OR = 0.078, 95%CI: 0.013-0.464) and mesalamine groups (50%; OR = 0.143, 95%CI: 0.025-0.819). The administration of adalimumab after intestinal resection was effective in preventing endoscopic and clinical recurrence of CD. Further larger studies are necessary to confirm the therapeutic advantage and to show the economic implications of biologic therapy in this field. Antibiotics, immunomodulatory medications and anti-TNF- α agents have been shown to be efficacious in preventing postoperative recurrence of CD, although the potential risks and benefits of therapy need to be balanced in individual patients.

CONCLUSION

In the management of jejunoileal CD, strictureplasty is an accepted surgical technique that relieves the obstructive symptoms, while preserving intestinal length and avoiding the development of short bowel syndrome. However, the role of strictureplasty in duodenal and colonic diseases remains controversial. In extensive colitis, after total colectomy with IRA, the recurrence rates and functional outcomes are reasonable. For patients with rectal involvement, total colectomy and end-ileostomy is safe and effective; however, a few patients can have subsequent IRA, and half of the patients will require proctectomy later. Proctocolectomy is associated with a high incidence of delayed perineal wound healing, but it carries a low recurrence rate. Patients undergoing proctocolectomy with IPAA had poor functional outcomes and high failure rates. However, if patients accept these risks and meet the selection criteria, such as isolated colonic CD without any evidence of terminal ileal or perianal involvement, there may be a role for IPAA.

Laparoscopic surgery has been introduced as a minimal invasive procedure. As compared with conventional surgery, laparoscopic procedures take longer to perform; however, patients who undergo laparoscopic surgery have a more rapid recovery of bowel function and a shorter hospital stay. There is no significant difference in the perioperative outcomes and the long-term reoperation rates between laparoscopic and conventional surgeries.

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Inflammatory bowel disease: Pathogenesis

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Abstract

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is characterized by chronic relapsing intestinal inflammation. It has been a worldwide health-care problem with a continually increasing incidence. It is thought that IBD results from an aberrant and continuing immune response to the microbes in the gut, catalyzed by the genetic susceptibility of the individual. Although the etiology of IBD remains largely unknown, it involves a complex interaction between the genetic, environmental or microbial factors and the immune responses. Of the four components of IBD pathogenesis, most rapid progress has been made in the genetic study of gut inflammation. The latest internationally collaborative studies have ascertained 163 susceptibility gene loci for IBD. The genes implicated in childhood-onset and adult-onset IBD overlap, suggesting similar genetic predispositions. However, the fact that genetic factors account for only a portion of overall disease variance indicates that microbial and environmental factors may interact with genetic elements in the pathogenesis of IBD. Meanwhile, the adaptive immune response

has been classically considered to play a major role in the pathogenesis of IBD, as new studies in immunology and genetics have clarified that the innate immune response maintains the same importance in inducing gut inflammation. Recent progress in understanding IBD pathogenesis sheds lights on relevant disease mechanisms, including the innate and adaptive immunity, and the interactions between genetic factors and microbial and environmental cues. In this review, we provide an update on the major advances that have occurred in above areas.

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Key words: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Pathogenesis; Genetics; Microbial factors; Immune responses

Core tip: Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis. Recent research indicated that the individual's genetic susceptibility, external environment, intestinal microbial flora and immune responses are all involved and functionally integrated in the pathogenesis of IBD. The main purpose of this review is to offer an update that have occurred in each of the above four areas, and to highlight the future work to find a clear understanding of IBD pathogenesis.

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INTRODUCTION

Inflammatory bowel disease (IBD) has been a global healthcare problem with a sustained increasing incidence^[1]. It includes two major forms, Crohn's disease (CD)

and ulcerative colitis (UC), which are distinct chronic bowel-relapsing inflammatory disorders. CD can cause transmural inflammation and affect any part of the gastrointestinal tract (most commonly, the terminal ileum or the perianal region) in a non-continuous type. Unlike UC, CD is commonly associated with complications such as abscesses, fistulas and strictures. In contrast, UC is typified by mucosal inflammation and limited to the colon^[2]. Although the etiology of IBD remains largely unknown, recent research indicated that the individual's genetic susceptibility, external environment, intestinal microbial flora and immune responses are all involved and functionally integrated in the pathogenesis of IBD^[3-5]. The main purpose of this review is to offer an update that have occurred in each of the above four areas, and to highlight the future work to find a clear understanding of IBD pathogenesis.

GENETICS

Over the past decades, there have been huge advances in our understanding of genetic contributions to IBD^[6]. This is due to the technological advances in DNA analysis and sequencing and the use of huge multinational databases^[7]. Advances in genetic testing and analyzing technologies have allowed for the completion of many genome-wide association studies (GWAS) which identify single nucleotide polymorphisms (SNPs). Recent studies have brought the number of IBD-associated gene loci to 163, of which 110 are associated with both diseases, 30 CD specific and 23 UC specific^[8]. Studies of gene loci shared by UC and CD may provide new way to find their common pathogenesis.

The era of modern IBD genetic research began in 2001 with the discovery of NOD2 (nucleotide-binding oligomerization domain-containing 2), the first susceptibility gene for CD^[9]. The NOD2 gene codes for a protein that was originally described as an intracellular receptor recognizing the muramyl dipeptide (MDP), a conserved motif present in peptidoglycan from both Gram-positive and -negative bacteria^[10]. MDP stimulation induces autophagy which controls bacterial replication and antigen presentation^[11,12], and modulates both innate and adaptive immune responses^[13]. NOD2 participates in distinct MDP-independent pathways such as the regulation of the T-cell response^[14]. The association between CD and NOD2 has already been replicated at the genome-wide significance level^[15].

Genetic analyses have shown an indispensable role for autophagy in immune responses in IBD, and reported two autophagy-related genes named *ATG16L1* and *IRGM*^[16-18]. Autophagy is involved in intracellular homeostasis, contributing to the degradation and recycling of cytosolic contents and organelles, as well as to the resistance against infection and removal of intracellular microbes^[19]. *ATG16L1* is essential for all forms of autophagy, and the coding mutation T300A is associated with an increased risk of CD. *IRGM* belongs to the p47

immunity-related GTPase family. CD-associated polymorphisms in *IRGM* lead to reduced protein expression. Epithelial cells and dendritic cells containing *ATG16L1* and NOD2 variants show defects in antibacterial autophagy^[12,20].

With the widespread use of GWAS and SNPs, a significant association between IBD and the *IL23R* gene has recently been described^[21]. The *IL23R* gene encodes a subunit of the receptor for the pro-inflammatory cytokine interleukin (IL)-23, a peptide involved in the generation of Th17 cells. The Th17 and IL-23 pathway is well established in the pathogenesis of IBD, with susceptibility gene loci *IL23R*, *IL12B*, *JAK2*, and *STAT3* having been identified in both UC and CD^[22,23]. Variants in *IL12B*, which encodes the p40 subunit of IL-12 and IL-23, have been associated with IBD and other immune disorders. Defects in the function of IL-10 have also been associated with CD and UC^[24]. Other susceptibility genes that regulate immune function include *CARD9*, *IL1R2*, *REL*, *SMAD3* and *PRDM1*.

Recent progress in the genetics of IBD holds several key messages in regard to the underlying mechanism of the disease. On one hand, the expanding number of susceptibility gene loci described in IBD indicates that genetic influences are critical components of the disease pathogenesis; while on the other hand, explainable susceptibility loci discovered so far account for only 20%-25% of the heritability found in the above-mentioned studies. This is not only true for IBD, but also true for many other polygenetic diseases, and the phenomenon has been called "the mystery of missing heritability of common traits" or "genetic vacuum"^[25]. This issue is further proved by GWAS that has failed to add new susceptibility gene loci to fill the "genetic vacuum"^[26,27]. The possibility was then proposed that, instead of missing genes, the interactions among genes and their products could explain much of the apparent vacuum and account for a considerable number of IBD^[25]. These new insights into genetics and heritability of IBD implicate that future explorations of gene-gene interactions, gene-pathway interactions and gene-environment interactions are likely to give us more insights into IBD pathogenesis than finding new rare variants.

ENVIRONMENT

There is no doubt that environmental factors play an important role in the pathogenesis of IBD. A large number of environmental factors are considered risk factors for IBD, including smoking, diet, drugs, geography, social stress, and psychological element^[28]. Among them, smoking remains the most widely studied and replicated environmental prompter for IBD. Since the first described inverse association between UC and smoking in 1982, subsequent studies have confirmed the protective effect of heavy smoking on the development of UC with a lower rate of relapse^[29-31]. Contrary to its effect on UC, smoking increases the risk of CD and is associated with a

higher rate of postoperative disease^[32].

Traditional conception for vitamin D's role is concentrated in calcium metabolism and bone health. Nowadays, there has been increasing recognition of the immunologic role of vitamin D^[33]. Recent literature suggests that the role of vitamin D is multifarious and associated with diverse diseases including IBD. Leslie *et al*^[34] found that vitamin D deficiency had been common in diagnosed IBD patients and pointed out that low vitamin D had contributed to the increased risk of IBD. In mouse models, vitamin D deficiency is associated with an increased susceptibility to dextran sodium sulfate-induced colitis and 1,25(OH)₂D₃ supplementation ameliorates the severity of intestinal inflammation^[35].

The effect of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) in the gastrointestinal tract is well recognized. However, limited high quality evidence is available to support the notion that aspirin and NSAIDs have an effect in triggering onset or relapse of IBD. Ananthakrishnan *et al*^[36] found no association between the dose, duration, or frequency of aspirin use and the risk for CD or UC; but the high dose, prolonged using duration, and frequent use of NSAIDs had been associated with an increased risk of CD and UC. A recent study has found that the use of antibiotics is an important environmental factor, influencing the risk of IBD through their effect on the microbiome. Antibiotic use within the first year of life is more common among pediatric IBD cases compared to controls^[37].

Stress has long been proposed to play a role in the pathogenesis of CD and UC^[38-40]. Bitton *et al*^[41] suggested that individuals with lower levels of stress had a reduced risk of the disease onset. Mood components of perceived stress, including depression and anxiety, may play a strong role in mediating the deterioration of IBD^[42]. A retrospective study by Goodhand *et al*^[43] found a reduction in the number of symptomatic relapses in participants, and the antidepressants beneficially impact the course of IBD. However, a Cochrane review shows no benefit of psychological interventions in IBD^[44].

Recent ecological and epidemiologic evidence suggests that air pollution may contribute to the risk of CD and UC. The rising incidence of CD and UC in developing countries parallels the development of industrialization^[45]. Elevated air pollution is associated with an augment in circulating polymorphonuclear leukocytes and plasma cytokines^[46,47]. Kaplan *et al*^[48] using The Health Improvement Network Database in the United Kingdom, found that high levels of NO₂ and SO₂ correlate with the increased risk of CD and UC. In another study, total pollutant emission has been linked to increased rates of hospitalizations for both CD and UC, suggesting that ambient air pollution may also influence these established diseases^[49].

MICROBIAL FACTORS

The whole human gut microbiome consists of ap-

proximately 1150 bacterial species, with each individual host having roughly 160 species^[50]. Gut microbiome is established within the first 2 wk of life and then usually remains remarkably stable thereafter. Although it is only possible to culture 20%-30% of the gut microbiome, the association between the changes in the microbiome and IBD has been established^[51]. Many studies have examined the gut flora in CD and UC in both inflamed and non-inflamed segments, and found that there is a significantly reduced biodiversity in faecal microbiome in IBD patients compared to that in healthy controls^[52]. Other research has also found that the microbiota in IBD patients is unstable than that in healthy individual^[53]. In healthy intestine, the Firmicutes and Bacteroidetes phyla predominate, and contribute to the production of epithelial metabolic substrates. In contrast, the microbiota is characterized by a relative lack of Firmicutes and Bacteroidetes, and an over-representation of enterobacteria in CD; meanwhile, a reduction in *Clostridium* spp. and an increase in *Escherichia coli* (*E. coli*) have been reported in UC^[54].

In healthy colon, there is a continuous mucus coating consisting of two layers of sub-structures: the outer is a loosely adherent layer, good for bacterial growth; while the inner is a tightly adherent layer, normally sterile. In IBD, particularly CD, there is a marked increase in bacteria associated with the colonic adherent mucus layer^[55,56]. In CD, a consistent increase in mucosa-associated *E. coli* and reduction in Firmicutes are reported^[57,58]. There is strong evidence for an increase in mucosa-associated *E. coli* in both the ileum and colon, and their presence within the granulomas in CD implicates a primary pathogenic role^[59]. An adherent and invasive *E. coli* (AIEC) phenotype has been found in CD, which is typified by bacterial invasion into epithelial cells and replication within macrophages^[60]. AIEC has also been shown to induce granuloma *in vitro* and granulomatous colitis in Boxer dogs^[61].

IMMUNOLOGICAL FACTORS

The investigation of IBD pathogenesis has been dominated for a long time by the studies of mucosal immunity, especially the T cell response. Available evidence suggests that the dysfunctions of innate and adaptive immune pathways contribute to the aberrant intestinal inflammatory response in patients with IBD. Most studies in the last two decades have focused on the role of abnormal adaptive immune responses in the pathogenesis of IBD. The focus on the adaptive immune response has ultimately led to the notion that the two main types of IBD represent clearly distinct forms of gut inflammation: CD has long been considered to be driven by a Th1 response and UC has been associated with a non-conventional Th2 response^[62,63]. The newly described Th17 cells are also involved in the gut inflammatory response in IBD^[64]. Immunological studies have recently focused on the mucosal innate immune responses, such as epithelial barrier integrity, innate microbial sensing, autophagy and unfolded protein response.

Innate immunity

The innate immune response represents our first line of defense against pathogens. It is non-specific, allowing the body to quickly respond to stimuli often within minutes or hours. The innate immune response is mediated by a large variety of different cell types including epithelial cells, neutrophils, dendritic cells, monocytes, macrophages and natural killer cells^[65]. This form of immunity is initiated by the recognition of microbial antigens, which is mediated by pattern recognition receptors including toll-like receptors (TLRs) on the cell surface and NOD-like receptors in the cytoplasm^[66]. Recent studies have found that the behavior of the cells mediating innate immunity and the expression and function of both TLRs and NOD proteins are altered significantly in individuals with IBD.

A British study shows that mucosal neutrophil accumulation and production of IL-1 β and IL-8 in response to trauma are selectively reduced in CD patients but not in UC patients^[67]. GWAS reveal that the NOD2 mutations most commonly associated with CD induce a defective ability of the gut to respond to LPS, and this defect may contribute to disease susceptibility^[68]. Although the functional role of NOD2 mutations is still controversial, available evidence suggests that they represent loss-of-function mutations that lead to reduced activation of NF- κ B^[69]. This inadequate response might result in reduced antibacterial agent production and pathogenic microbial invasion^[70]. Other studies suggest that the loss of function of NOD2 may result in the lack of inhibition of TLR2 stimulation, leading to activation of inflammatory pathways and excessive Th-1 responses^[71]. Furthermore, NOD2 also contributes to immune tolerance. These effects are impaired in cells from patients with NOD2 mutation 3020insC^[72].

IL-23 is a key cytokine both in innate and adaptive immunity and possesses a central role in driving early responses against microbes. IL23R polymorphisms have been associated with both CD and UC, suggesting that IL-23 may represent a shared inflammatory molecule in chronic intestinal inflammation. Recent studies have shown that, besides its activity on Th17 cells, IL-23 can also act on cells of the innate immune system. IL-23 has been shown to induce Th17 cytokine production by innate lymphoid cells (ILCs) that share the phenotype of lymphoid tissue-induced cells^[73].

CD has also been associated with *ATG16L1* and *IRGM* genes, which are involved in autophagy. *ATG16L1* is essential for all forms of autophagy, and the coding mutation T300A is associated with an increased risk of CD. Autophagy is one of the mechanisms for maintaining cellular homeostasis and considered very important for host defense against intracellular microorganisms. Normally, autophagy is induced by bactericidal effects and presentation of endogenous antigens, and these processes are impaired in patients with mutations in NOD2 or *Atg16L1*. Closely relating to autophagy and innate immunity, dysregulation of the unfolded protein response

may also contribute to IBD pathogenesis. This response is induced by endoplasmic reticulum stress and finally induces apoptotic cell death and causes IBD^[74].

In addition, defective epithelial barrier and increased intestinal permeability have long been observed in IBD patients^[75]. The first physical barrier that intestinal bacteria and food antigens encounter on the mucosal surface is represented by the mucous layer that covers the intestinal epithelium. The importance of mucus in the prevention of bacterial break-through and intestinal inflammation has been proved by many studies^[76]. The second line of defense against bacterial invasion is formed by the intestinal epithelium which consists of enterocytes and specialized epithelial cells, such as goblet cells and Paneth cells. Besides forming a physical barrier against bacteria, epithelial cells can secrete a number of antimicrobial peptides. Defective expression of antimicrobial peptides has been observed in patients with CD^[77].

Adaptive immunity

As opposed to the innate immune response, the adaptive immunity is highly specific, often takes several days to respond and depends on the type and number of T cells. Th1 cells, induced by IL-12, produce a high amount of IFN- γ , whereas Th2 cells release IL-4, IL-5 and IL-13^[78]. An abnormal Th1 immune response is thought to cause intestinal inflammation in CD, and it has been observed that mucosal T cells from CD patients produce higher amounts of IL-2 and IFN- γ than T cells from UC patients or controls do^[79]. It has also been shown that in UC, atypical NK T cells release higher amounts of the Th2 cytokine IL-13 than T cells from controls or CD patients do^[80,81]. Therefore, CD has been thought to be characterized by a Th1 immune response, while UC has been considered as a Th2-mediated disease^[82]. However, there have also been different observations about mucosal Th1 and Th2 cytokines in IBD. Both UC and CD biopsies cultured *in vitro* release high and comparable amounts of IFN- γ ^[83]. Lower levels of IL-13 are found in the colonic mucosa of UC patients compared to those in CD patients and subjects of the control group. Recent studies on experimental colitis have suggested an anti-inflammatory effect of IL-13 in the gut^[84,85]. It has been also observed that IL-13 levels in the supernatants of intestinal biopsies cultured *in vitro* are lower than IFN- γ concentration and the concentrations are comparable among CD, UC and control groups^[86]. Similar observations have been reported by Bernardo *et al.*^[87] who have described the presence of a mixed cytokine profile with predominance of IL-6 and the absence of IL-13 in supernatants of UC biopsies cultured *in vitro*. Collectively, these data should lead us to reconsider the Th1/Th2 paradigm in CD and UC^[82], but the conception that UC is a Th2-mediated disease remains controversial.

Th17 cells are a T cell subset characterized by the production of large amounts of IL-17A, IL-17F, IL-21 and IL-22. They are induced by a combination of IL-6 and transforming growth factor (TGF)- β , and their ex-

pansion is promoted by IL-23^[88]. The involvement of Th17 cells and, in particular, their signature cytokine IL-17A in intestinal inflammation has been extensively studied. High transcript levels of IL-17A have been detected both in CD and UC mucosa in comparison to normal gut^[89,90]. Moreover, the inflamed IBD mucosa cultured *in vitro* produces higher levels of IL-17A than the control^[79]. Furthermore, Th17 cells are an important source of IL-21, an IL-2-related cytokine which is up-regulated in inflamed IBD mucosa^[91,92]. The true role of Th17 cells in IBD pathogenesis is currently undergoing intense scrutiny, and it is particularly fascinating that Th17 cells express the IL-23R on their surface^[93].

CONCLUSION

There is no doubt that an unprecedented progress in our understanding of IBD pathogenesis has been achieved during the past few years. The key factors responsible for IBD include genetic components, environmental elements, microbial flora and immune responses. It is hard to dispute the popular belief that IBD arises from an extremely complex interaction among genetic and environmental elements, dysregulated immune responses and alterations of the microbiome, and that none of these factors alone is likely to cause the disease. More detailed information on their composition, function, and interaction is becoming increasingly accessible through high genomic approaches, investigation of environmental changes, molecular analysis of gut bacteria flora, and a more integrated understanding of the interaction between innate and adaptive immune responses. The growing number and diversity of genetic loci associated with IBD provide major challenges to the investigation of how they impact immunity and inflammation in susceptible individuals. Future research needs to further clarify and integrate the effects of the microbiome and environment on the immune response, and it shall be essential to gain further insights into the mechanisms and pathways of how bacteria, viruses or even fungi can modulate innate and adaptive immune responses.

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Pulmonary manifestations of Crohn's disease

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Key words: Crohn's disease; Inflammatory bowel disease; Lung; Extracolonic involvement

Core tip: The clinicopathological patterns of pulmonary involvement consist of subclinical alterations, airway diseases, lung parenchymal diseases, pleural diseases and drug-related diseases in Crohn's disease (CD). The treatment of CD-related respiratory disorders depends on the specific pattern of involvement, and in most patients, steroids are required in the initial management. This review focuses on the pulmonary manifestations of CD in an attempt to avoid further impairment of health status and to alleviate patient symptoms by prompt recognition and treatment.

Abstract

Crohn's disease (CD) is a systemic illness with a constellation of extraintestinal manifestations affecting various organs. Of these extraintestinal manifestations of CD, those involving the lung are relatively rare. However, there is a wide array of lung manifestations, ranging from subclinical alterations, airway diseases and lung parenchymal diseases to pleural diseases and drug-related diseases. The most frequent manifestation is bronchial inflammation and suppuration with or without bronchiectasis. Bronchoalveolar lavage findings show an increased percentage of neutrophils. Drug-related pulmonary abnormalities include disorders which are directly induced by sulfasalazine, mesalamine and methotrexate, and opportunistic lung infections due to immunosuppressive treatment. In most patients, the development of pulmonary disease parallels that of intestinal disease activity. Although infrequent, clinicians dealing with CD must be aware of these, sometimes life-threatening, conditions to avoid further impairment of health status and to alleviate patient symptoms by prompt recognition and treatment. The treatment of CD-related respiratory disorders depends on the specific pattern of involvement, and in most patients, steroids are required in the initial management.

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INTRODUCTION

Crohn's disease (CD) is a granulomatous systemic disorder of unknown etiology commonly involving the gastrointestinal tract. However, CD may also have extraintestinal manifestations, which occur in at least 25% of CD patients^[1]. Of these extraintestinal manifestations, arthritis, erythema nodosum, pyoderma gangrenosum, and primary sclerosing cholangitis are the most common. The lungs are not classically thought to be affected, although there is growing evidence for pulmonary involvement in CD^[2-11]. CD can involve the tracheobronchial tree, the lung parenchyma and the pleura^[12]. Although obvious pulmonary involvement is exceptional, latent pulmonary impairment and subclinical alveolitis as evidenced by lymphocytosis in bronchopulmonary lavage (BAL) have been described and are well recognized^[13,14].

There are a number of mechanisms by which the lungs may become involved in CD. These include the same embryological origin of the lung and gastrointestinal tract by ancestral intestine^[15], similar immune systems in the pulmonary and intestinal mucosa^[16], the presence of circulating immune complexes and auto-antibodies^[17], and the adverse pulmonary effects of some drugs.

CD is characterized by an exaggerated immune response to the luminal flora, suggesting that deficiencies in barrier function of intestinal flora may be involved^[18,19]. The epithelial layer of the intestines must meet two opposing requirements: on one hand it must allow for efficient uptake of nutrients and fluids, and on the other hand it is a vital defense barrier between the milieu interior and the milieu exterior. Airway epithelia contain a cell-autonomous system in which motile cilia both sense noxious substances entering airways and initiate a defensive mechanical mechanism to eliminate the offending compound^[20]. In contrast to the lung which by virtue of ciliary movement is kept virtually sterile, the gut epithelium is confronted by a large microbiological load and a substantial xenobiotic challenge^[21]. This may explain why lung involvement is quite rare in CD.

The clinicopathological patterns of pulmonary involvement consist of subclinical alterations, airway diseases, lung parenchymal diseases, pleural diseases and drug-related diseases. The present article examines pulmonary manifestations of CD.

CD-RELATED LUNG DISEASES

Subclinical alterations

Although the overall prevalence of concomitant bronchopulmonary manifestations is only 0.4%^[22], subclinical alterations in at least half of adults with CD have been demonstrated^[23-25], suggesting the underlying bronchial inflammation. Patients with CD present with a subclinical inflammatory process despite the absence of pulmonary symptoms^[26]. This pulmonary involvement can be reflected by an increased lymphocyte count in the BAL fluid^[27,28] and/or lung function abnormalities^[29,30].

BAL has provided a fresh dimension in the investigation of pulmonary and multisystem disorders. BAL fluid may be analyzed for cells and chemical mediators in the diagnosis and serially in the management of granulomatous disorders such as CD^[31]. BAL studies in asymptomatic CD subjects have demonstrated the presence of persistently elevated alveolar lymphocytosis, suggesting latent pulmonary involvement^[23,32]. There is no correlation between BAL differential cell count and drug treatment or CD site, and activity^[27].

Pulmonary function test abnormalities are frequently found in patients with CD without the presence of respiratory symptoms or lung radiograph findings^[33]. The severity and frequency of these pulmonary function test abnormalities which are detected even in remission periods increase with activation of the disease^[34]. Pulmonary inflammation may correlate with bowel inflammation, as

shown in the studies^[23,35,36] which demonstrated a reduction in diffusing capacity and other pulmonary function abnormalities during CD exacerbations. Moreover, lung transfer factor for carbon monoxide (TLCO) abnormalities are related to the degree of disease activity^[35]. Therefore, pulmonary function tests may be used as a non-invasive diagnostic procedure to determine the activation of CD and might aid the early diagnosis of latent respiratory involvement.

Nitric oxide (NO) can be detected non-invasively in exhaled air (eNO) and is considered a surrogate marker of airway inflammation. Fractional eNO values were found to be significantly higher in CD patients and correlated positively with CD activity. eNO measurement may be of clinical value in the follow-up of CD patients^[37]. An increased eNO level may be used to identify patients with CD who need further pulmonary evaluation^[38]. It is important to be alert to this clinical disorder and to try to detect it as early as possible in order to prevent future respiratory disturbances.

Airway diseases

CD is an inflammatory bowel disease associated with a variety of systemic manifestations, including large and small airway involvement. Major patterns of airway diseases associated with CD are upper-airway obstruction^[39-41], tracheobronchitis^[42,43], chronic bronchitis^[44], granulomatous bronchiolitis^[45], bronchiectasis^[41], asthma^[46] and acute respiratory failure due to tracheobronchial involvement^[47]. In cases with large airway involvement, marked tracheobronchial inflammation and narrowing of the tracheal and/or bronchial lumen are typically observed at bronchoscopy as erythematous and edematous tracheal mucosa with diffuse scattered whitish lesions, while biopsy reveals metaplastic changes in the epithelium, granulomatous infiltration by inflammatory cells and mucosal ulcerations^[7,42,47]. The latter is most often a subclinical condition, and requires expensive and invasive diagnostic approaches. Bronchial hyperresponsiveness may be the expression of subclinical inflammation of the airways by several inflammatory cell types and their products, epithelial damage, microvascular leakage, and autonomic neural mechanisms^[48], a phenomenon which can be responsible for the development of various pulmonary manifestations in CD^[49].

The most commonly reported airway disease is bronchiectasis^[50,51], which is defined as an abnormal and irreversible dilation of the medium-sized bronchioles. It most frequently presents with cough and copious amounts of sputum production. In some patients, the manifestations of bronchiectasis may only become clinically significant after surgery and the withdrawal of medical treatment^[52]. Bronchiectasis is commonly associated with childhood pneumonia, necrotizing pneumonia, bronchial obstruction, and diseases that cause abnormal host immunity.

Tracheobronchitis associated with CD has several very specific clinical findings^[42,53]. A productive dry

cough is typically the chief symptom, occasionally associated with shortness of breath or fever. It can often be identified by history, complemented by a clear X-ray film and obstructive pattern on pulmonary function testing. Although X-ray films of the chest field are usually normal, inflammation of the peripheral airways may present as infiltrates. Bronchoscopy shows diffuse inflammation of the trachea and bronchi with diffuse scattered whitish lesions, while biopsy reveals metaplastic changes in the epithelium and granulomatous infiltration by inflammatory cells.

The treatment of CD-related airway diseases depends on the specific pattern of involvement, and if left untreated, the patient will be put at risk of developing irreversible destruction of the air passage^[54]. In the majority of patients with airway diseases, marked and long-lasting responses are seen following systemic or inhaled steroids. Bronchial lavages with methylprednisolone are effective in some patients with severe airway inflammation.

Lung parenchymal diseases

Several forms of lung parenchyma involvement in CD are recognized, including interstitial lung diseases such as bronchiolitis obliterans with organizing pneumonia (BOOP)^[55,56], unspecified interstitial lung disease^[57-59], non-caseating granulomatous inflammation and fibrosis^[60], parenchymal nodules and granulomata^[61-63], alveolitis^[64] and alveolar consolidation^[65]. *Mycobacterium xenopi* infection^[66], noninfectious lung pathology^[67], colopleural fistula and fecopneumothorax^[68,69] have also been described in CD.

Cryptogenic organizing pneumonia, formerly known as BOOP, often caused by inhalation injury, or from a post-infection origin or drugs, has been described in about a dozen cases of CD, and may present acutely or sub-acutely with fever, cough, dyspnea and pleuritic chest pain^[70]. Radiographic findings may range from patchy focal opacities to diffuse infiltrates on plain films, to pleural opacities and air bronchograms on chest computed tomography (CT) scans.

Although interstitial diseases most commonly involve drug-induced reactions with mesalamine and sulfasalazine, a small number of unrelated cases of fibrosing alveolitis and eosinophilic pneumonia have been reported^[59]. In patients with an interstitial lung disease, most require open or thoracoscopic lung biopsy for diagnosis and clarification of the disease. The latter technique may be useful for precise diagnosis with minimal invasion. The alterations are similar, showing acute alveolitis, granulomatous lymphocytic infiltration of the interstitium and of the walls of small arteries, with slight interstitial fibrosis. Sarcoidosis is included in the differential diagnosis of these lesions in some cases.

CD and sarcoidosis are chronic inflammatory barrier diseases that share several common clinical, genetic and immunological features^[70], including the occurrence of granulomas. Since these two conditions also share common susceptibility loci^[71], it is not surprising that these two diseases may simultaneously appear in the same

patient, with pulmonary involvement^[72], although this happens quite rarely and the two diseases usually follow an independent clinical course^[73]. The clinical pictures of these two diseases are usually easy to differentiate, due to the topography of the lesions: while both diseases may be disseminated, sarcoidosis mainly involves mediastinal lymph nodes and lungs, while CD is essentially a digestive disease.

Multiple pulmonary nodules are an infrequent finding in patients with CD. When they are found, the nodules are composed of sterile aggregates of neutrophils with necrosis, and histology usually shows sterile necrobiotic nodules, which are spherical, and aggregates of neutrophils, which frequently cavitate^[62].

Fistula formation is frequent in CD and occurs in approximately 33% of patients^[74]. However, fistulous communication between the pleural cavity and adjacent organs below the diaphragm is an extremely rare complication of CD. Recurrent pneumonia with feculent sputum in patients with CD should raise suspicion of colobronchial fistula. The diagnosis of fecopneumothorax is based on meticulous clinical examination and additional diagnostic procedures. Abdominal and thoracic CT scans or magnetic resonance imaging (MRI) may provide additional information on the stage of the disease and can exclude the presence of abscess or fluid collection in the abdominal cavity. Colopleural fistula and fecopneumothorax are rare, but life-threatening complications of CD^[75]. Surgical treatment is mandatory as soon as the diagnosis is established^[76].

The manifestations of lung parenchyma in CD usually respond markedly to inhaled and/or systemic steroids. Steroids administered orally lead to marked improvement in patients with interstitial lung disease and necrotic nodules, and intravenous steroids are required in the initial management of life-threatening complications such as extensive interstitial lung disease. The addition of cyclophosphamide or infliximab may result in a rapid clinical and radiologic response and is well tolerated in some cases^[77,78].

Pleural diseases

Few cases of pleural involvement in CD have been reported in the literature. Pleural involvement can be classified as: pneumothorax^[79], pleural thickening^[80], pleuritis and pleural effusion^[81,82]. Pleural effusion alone is a rare manifestation and is more often associated with pericarditis^[45]. Pleural fluid is an exudate containing neutrophils and may be hemorrhagic. The pleural complications of CD may run an independent course and may be present at the time of inactive bowel disease. Mesalamine may also induce lupus-like symptoms, such as arthralgia, pericarditis, tamponade, and/or pleural effusion, with positive antinuclear antibodies^[83]. Therefore, pleural diseases induced by drugs need to be ruled out. Prednisone is administered for pleural complications if the patient is not already on a regimen of this drug or an increased dosage of prednisone is given, which usually results in resolution

of the pleural effusions. However, pleural drainage may occasionally be required.

DRUG-RELATED LUNG DISEASES

Although drug-related diseases are not “proper” CD-associated diseases, as CD patients use several drugs for prolonged periods of time, it is not surprising that some of these may also cause problems to the lungs; therefore, this type of pathology must be kept in mind in patients taking sulfasalazine, mesalamine, methotrexate, and anti-tumor necrosis factor (TNF)-alpha.

Sulfasalazine and mesalamine

Sulfasalazine and mesalamine are commonly used medications for the long-term treatment of CD, and their side effects may be dose-related or idiosyncratic and should be differentiated from the respiratory involvement occurring in CD and due to the underlying disease, although this is challenging because they share similar pathological features^[45]. Commonly reported lung pathology related to the use of these compounds is mostly due to interstitial disease^[84-88], although eosinophilic pleuritis^[89] and eosinophilic pneumonia^[13,90,91] have also been described. Patients present with progressive respiratory symptoms such as dyspnea, chest pain and cough and radiographic abnormalities. Alternatively, sulfasalazine and mesalamine may induce asymptomatic lung injury more commonly than is presently suspected^[92]. In most cases, symptoms appear after 2-6 mo of drug use, whereas in a few cases they appear after some days or after many years^[93]. Interestingly, these pulmonary toxicities appear reversible after withdrawal of the drug, and in some cases, with the use of systemic corticosteroids^[14].

Azathioprine and 6-Mercaptopurine

Azathioprine (AZA) and 6-Mercaptopurine (6-MP) are therapeutic options for patients with moderate to severe CD^[94]. Pulmonary toxicity due to these drugs has been reported infrequently in the literature, although interstitial pneumonitis, BOOP^[95], chronic pneumonitis/fibrosis and pulmonary edema^[96] have been described after use of AZA and 6-MP. Although rare, AZA and 6-MP can cause direct, dose-dependent and serious pulmonary toxicity^[95,97]. The largest series of lung toxicity related to AZA was described in 7 cases undergoing renal allograft transplant immunosuppression with AZA^[97]. Lung biopsies revealed interstitial pneumonitis in 5 patients and diffuse alveolar damage in 2 patients; 3 patients died and the other 4 improved after stopping AZA and in 2 of these patients cyclophosphamide therapy was needed to completely resolve this side effect. Thus, it is important for clinicians to have a high index of suspicion for this adverse reaction which occurs within 1 mo after purine analog use in CD.

Methotrexate

Methotrexate (MTX) may be useful in the treatment of

CD^[98], but can cause adverse effects in the lungs, which in some cases are lethal^[99]. The mechanism of MTX-induced lung pathology remains unclear. A hypersensitivity reaction was suggested by lung biopsy findings: interstitial pneumonitis, granuloma formation and bronchiolitis^[100], and by BAL findings: lymphocytic alveolitis, increased eosinophils and reversed CD4/CD8 ratio^[101], together with the clinical findings of fever, peripheral eosinophilia and response to corticosteroids. MTX may also cause pneumonitis^[102] and abnormal ventilation is an early sign and should lead to further investigation^[103]. The diagnosis of MTX-induced lung disease is difficult as there are no pathognomonic findings and this condition may mimic other pulmonary diseases. The most frequent complaints include dyspnea, fever and nonproductive cough. Lung function tests show a restrictive picture with low carbon monoxide diffusion capacity. As MTX-related lung toxicity is potentially fatal, regular monitoring of the status of the respiratory system in MTX-treated patients is necessary and patients should be instructed to report any new pulmonary symptoms without delay^[104]. Besides supportive therapy, withdrawal of MTX seems a logical approach.

Biological therapy

Biological therapy with anti-TNF drugs such as infliximab, adalimumab and certolizumab has represented a significant advance in the treatment of CD over the past few years^[105-108]. However, serious side effects do occur, necessitating careful monitoring of therapy^[109]. A number of associated opportunistic infections have been observed as a result of suppression of T cell-mediated immunity, the most frequent being tuberculosis^[110-112]. Physicians should be aware of the increased risk of reactivation of tuberculosis in patients treated with anti-TNF agents and regularly look for usual and unusual symptoms of tuberculosis. Moreover, the use of biological therapy has been associated with *Pneumocystis carinii* pneumonia^[113], as well as with other pulmonary infections (coccidiomycosis, histoplasmosis, aspergillosis, nocardia asteroides, actinomycosis and listeriosis)^[114-118], especially in older patients^[119].

Although infective complications are the most feared after the use of biological agents, these may induce other uncommon effects in the lung, such as acute respiratory distress syndrome^[120], diffuse alveolar hemorrhage^[121], nonbronchiolitis inflammatory nodular pattern of the lung^[122] and interstitial lung disease^[123-126]. Close observation of patients undergoing treatment with TNF inhibitors for evolving signs and symptoms of autoimmunity is required. Organ involvement is unpredictable, which makes correct diagnosis and management extremely challenging^[127].

CONCLUSION

In conclusion, CD is a systemic disorder and not restricted to the intestine. Pulmonary manifestations of

CD are being increasingly recognized. The involvement of the respiratory system is relatively rare, but sometimes potentially harmful. The lung manifestations of CD vary and often represent a confounding diagnostic problem necessitating a complex work-up. As far as possible, extraintestinal manifestations need to be distinguished from the complications of intestinal inflammation and from the side effects of drugs used in its treatment. Patients suffering from CD should undergo pulmonary evaluation which should include physical examination, chest X-ray and pulmonary function tests with measurement of diffusing capacity of carbon monoxide. Invasive measures, such as bronchoscopy and thoracoscopy, are typically required to reach a final diagnosis and steroids are the most frequently reported treatment. It is imperative to maintain a high index of suspicion for the development of pulmonary disease in the setting of CD in order to initiate appropriate early treatment and avoid complications.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Novel methylxanthine derivative-mediated anti-inflammatory effects in inflammatory bowel disease

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inducers. Anti-inflammatory effects of methylxanthine derivatives have been well-documented in the literature. For example, a beneficial link between coffee or caffeine consumption and type 2 diabetes as well as liver cirrhosis has been reported. Furthermore, theophylline has a long history of being used as a bronchodilator in asthma therapy, and pentoxifylline has an immuno-modulating effect for peripheral vascular disease. However, it is still largely unknown whether these methylxanthine derivative-mediated anti-inflammatory effects are associated with the inhibition of CHI3L1-induced cytoplasmic signaling cascades in epithelial cells. In this review article we will examine the above possibility and summarize the biological significance of methylxanthine derivatives in intestinal epithelial cells. We hope that this study will provide a rationale for the development of methylxanthine derivatives, in particular caffeine, -based anti-inflammatory therapeutics in the field of IBD and IBD-associated carcinogenesis.

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Abstract

Family 18 chitinases have a binding capacity with chitin, a polymer of N-acetylglucosamine. Recent studies strongly suggested that chitinase 3-like 1 (CHI3L1, also known as YKL-40) and acidic mammalian chitinase, the two major members of family 18 chitinases, play a pivotal role in the pathogenesis of inflammatory bowel disease (IBD), bronchial asthma and several other inflammatory disorders. Based on the data from high-throughput screening, it has been found that three methylxanthine derivatives, caffeine, theophylline, and pentoxifylline, have competitive inhibitory effects against a fungal family 18 chitinase by specifically interacting with conserved tryptophans in the active site of this protein. Methylxanthine derivatives are also known as adenosine receptor antagonists, phosphodiesterase inhibitors and histone deacetylase

Key words: Adherent-invasive *Escherichia coli*; Chitinase 3-like 1; Chitinase inhibitors; Intestinal epithelial cells; Host-microbial interactions; Inflammatory bowel disease

Core tip: The involvement of family 18 chitinases in the pathogenesis of inflammatory bowel disease has been increasingly characterized. The discovery of methylxanthine derivatives as an effective inhibitor of family 18 chitinases provides a good tool to control the pathogenic effects of these proteins. This review discusses the underlying inhibitory mechanisms of the different methylxanthine derivatives and how these compounds have been shown to be effective in the amelioration of animal colitis models. As such, this mode of application can be extended to target other family 18 chitinases associated disorders such as asthma.

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INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a group of intestinal inflammatory disorders that affect millions people worldwide. IBD is associated with increased risk of colorectal cancer 8-10 years after initial diagnosis^[1]. The chronic colitis in IBD is associated with inappropriate activation of the immune system by abnormal interactions between host and enteric luminal microbes. Our group have previously identified an unexpected role for chitinase 3-like 1 (CHI3L1) in enhancing bacterial adhesion and invasion on/into intestinal epithelial cells (IECs) and have demonstrated that CHI3L1 specifically activates protein kinase B (AKT) phosphorylation in IECs^[2,3]. Given these roles, the ability of a host to produce CHI3L1 and other enzymatic active mammalian chitinases [*e.g.*, chitinase-1 and acidic mammalian chitinase (AMCase)] could be a critical factor in regulating the innate immune responses against microorganisms that exist in normal intestinal flora^[4]. However, exaggerated production of these chitinases could cause highly pathogenic effects in mucosal tissues, directly initiating and perpetuating chronic inflammation^[2,5-7]. CHI3L1 also has been identified as a potential autoantigen driving T cell-mediated immune responses in rheumatoid arthritis, suggesting that mammalian chitinases are highly associated with chronic inflammation^[8,9].

As shown by Rao *et al.*^[10], methylxanthine derivatives, including caffeine, theophylline and pentoxifylline, are competitive inhibitors against a family 18 chitinase expressed by a fungal pathogen. Crystallographic analysis of chitinase and methylxanthine derivative complexes revealed specific interactions with the active site of the chitinase protein, mimicking the binding of allosamidin, a well-known pan-chitinase inhibitor isolated from *Streptomyces* species^[10]. Currently, most known family 18 chitinase inhibitors are natural products, including pseudo-trisaccharide allosamidin^[11]. However, this inhibitor is unsuitable as a therapeutic lead because of its high cost and high molecular weight. In contrast, methylxanthine derivatives are inexpensive and have much lower molecular weight as compared to allosamidin. In particular, caffeine is found in a wide variety of foods and beverages (*e.g.*, coffee, tea, cola, chocolates) and dietary supplements/ingredients (including botanicals such as guarana, yerba mate, and green tea extract)^[11]. At physiological concentration, caffeine shows only minor adverse effects on the cardio-respiratory system and other health outcomes^[12-14]. Therefore, caffeine is thought to be the most

reasonable, least expensive, and safest compound among known chitinase inhibitors. In fact, our group recently demonstrated the beneficial effects of a medium dose of caffeine (2.5 mmol/L; equivalent to the concentration of caffeine in 2-3 cups of coffee) in the development of acute dextran sulfate sodium (DSS)-induced colitis by down-regulating the expression of CHI3L1 in the colon^[15]. Although anti-inflammatory effects of caffeine is considered to be mediated, at least partially, *via* chitinase inhibition, it is still largely unknown whether the other methylxanthine derivatives, such as theophylline and pentoxifylline, also exert their anti-inflammatory activities by downregulating CHI3L1 expression. In this review article, we will discuss the important biological functions of caffeine, theophylline and pentoxifylline laying a special emphasis on the CHI3L1-mediated AKT/ β -catenin signaling activation in IECs.

CHI3L1, BACTERIAL INFECTION AND IBD

It has been postulated that dysregulated host-microbial interactions play a central role in the development of intestinal inflammation^[16-18]. In humans, the ileocecal region and colon are colonized by a group of anaerobic bacteria, many of which cannot be cultured using standard microbial techniques^[19]. Altered epithelial barrier functions, mucosal immune responses and microbial defense are major factors of host susceptibility against these commensal bacteria^[19]. Therefore, abnormal adhesion and invasion of commensal bacteria on/into IECs may be highly involved in the pathogenesis of IBD in patients with the mutations in IBD-susceptibility genes^[20,21]. The development of excess bacterial adhesion and/or perpetuation of intestinal inflammation seems to be closely associated with the induction of several molecules on IECs^[22,23].

Previous studies have addressed the possibility that chronic bacterial infections are involved in the pathogenesis of IBD^[24-26]. An involvement of *Escherichia coli* (*E. coli*) in the pathogenesis of CD has been suggested by the detection of *E. coli* antigens and DNA in granulomatous and peri-ulcerative lesions in CD^[27]. In addition, circulating antibodies against the porin protein C of *E. coli* outer membrane have been detected in CD patients with severe inflammation^[28]. In fact, the terminal ileum of CD patients is sometimes heavily colonized by a special type of *E. coli* strain, adherent-invasive *E. coli* (AIEC), which is able to survive extensively within IECs and macrophages without inducing apoptosis^[29-32]. Interestingly, AIEC can be detected only in 6% of ilea in healthy individuals, but is present in 36% of the newly formed terminal ilea (with early and acute inflammation) of post-surgical patients^[31]. It has been demonstrated by Carvalho *et al.*^[33] that abnormal expression of specific host receptor, carcinoembryonic antigen-related cell adhesion molecule 6, is one of the inducible molecules enhancing the interaction between host cells and AIEC^[32,33].

Utilizing DNA microarray analysis, our group also identified that CHI3L1 is specifically up-regulated on

Table 1 Methylxanthines compared with common chitinase inhibitors in IC₅₀

Compound name	Chemical formula	IC ₅₀	Ref.
Caffeine	C ₈ H ₁₀ N ₄ O ₂	469 ± 23 μmol/L against <i>Aspergillus fumigatus</i> (A. <i>fumigatus</i>) chitinase	[10]
Pentoxifylline	C ₁₃ H ₁₈ N ₄ O ₃	126 ± 7 μmol/L against A. <i>fumigatus</i> chitinase	[10]
Theophylline	C ₇ H ₈ N ₄ O ₂	1500 ± 90 μmol/L against A. <i>fumigatus</i> chitinase	[10]
Allosamidin	C ₂₅ H ₄₂ N ₄ O ₁₄	10 μmol/L against <i>Candida albicans</i> chitinase	[42]
Argifin	C ₂₉ H ₄₁ N ₉ O ₁₀	3.7 μmol/L against <i>Lucilia cuprina</i> (L. <i>cuprina</i>) chitinase	[78]
Argadin	C ₂₉ H ₄₂ N ₁₀ O ₉	3.4 nmol/L at 20 °C against L. <i>cuprina</i> chitinase	[79]

IC₅₀: The half maximal inhibitory concentration.

IECs under intestinal inflammatory conditions. Although CHI3L1 entirely lacks glycohydrolase enzymatic activity, it has a functional chitin-binding motif acting as chitin^[34,35]. Chitin is an *N*-acetylglucosamine polymer and is the second most abundant polysaccharides in nature next to cellulose. In spite of lacking of chitin and chitin synthase, mammals can constitutively or inducibly produce several chitinases, including CHI3L1, which show a high degree of sequential homology to the bacterial and plant chitinases^[36]. The expression of CHI3L1 is highly up-regulated in IECs and macrophages with inflammation and specifically enhances potentially pathogenic, but not non-pathogenic, bacterial adhesion and invasion on/into IECs^[2,37]. Our recent studies further revealed that a specific adhesion between CHI3L1 and 5 distinct amino acids in the AIEC Chitinase A (ChiA) protein, which includes chitin-binding domains (CBDs), play critical roles in the initial host-microbial interaction^[7]. Furthermore, *N*-glycosylation of a single amino acid residue (68th Asparagine) in the mouse CHI3L1 protein is crucial for the adhesion of potentially pathogenic *E. coli* on IECs^[7]. Interestingly, similar to CHI3L1, bacterial CBDs have been found to bind directly to chitin^[38,39]. Therefore, the specific interaction between glycosylated CHI3L1 and *E. coli* ChiA seems to be enhancing the bacterial adhesion and invasion on/into IECs under inflammatory conditions. These excess and abnormal host-microbial interactions *via* the above two chitinases may further perpetuate chronic intestinal inflammation as well as colitis-associated carcinogenic change of IECs, presumably by interacting with toll-like receptor-4 signaling^[40,41].

METHYLXANTHINE DERIVATIVES AS PAN-CHITINASE INHIBITORS

Methylxanthines are a group of alkaloid chemicals which are derived from the purine base xanthine. Xanthine is a result of purine degradation from either guanine by guanine deaminase or hypoxanthine by xanthine oxido-

reductase. Methylxanthines are methylated derivatives and include the compounds caffeine, aminophylline, 3-isobutyl-1-methylxanthine, paraxanthine, pentoxifylline, theobromine (found in chocolate), theophylline. Traditionally, they are used as stimulants, to increase athletic performance, and as bronchodilators, most notably in the case of asthma.

Through the use of drug screening tools, it was demonstrated that several methylxanthine derivatives, namely caffeine, theophylline, and pentoxifylline were potential chitinase inhibitors^[10]. Subsequent analysis confirmed the inhibitory effects of all 3 methylxanthines, with pentoxifylline having the highest K_i of 37 μmol/L. In terms of another parameter IC₅₀ (half maximal inhibitory concentration), pentoxifylline was almost 4 fold lower, and thus 4 times more effective, than caffeine (126 μmol/L *vs* 469 μmol/L, respectively), and was almost 12 times more powerful than theophylline (1500 μmol/L)^[10]. In contrast, allosamidin is reported of having an IC₅₀ of 10 μmol/L towards *Candida albicans*-derived chitinase^[42]. Therefore, methylxanthines including pentoxifylline, caffeine and theophylline do show a significantly lower affinity against fungal chitinase as compared to allosamidin. The inhibitory strength of methylxanthines compared with common chitinase inhibitors is summarized in Table 1.

Interestingly, the chemical structures involved in binding between methylxanthines and the family 18 chitinases were found to be very similar for all 3 compounds (caffeine, pentoxifylline and theophylline) and mimicked chitinase binding to allosamidin. X-ray diffraction analysis revealed a common position for the methylxanthine substructure. The additional inhibition by pentoxifylline is suspected to be due to increased interactions including hydrogen bonding, and extensive π-π stacking with the active site^[10].

Methylxanthines have the potential to be very useful as chitinase inhibitors and disease treatments as summarized in Table 2. They are typically very safe in low doses and represent a class of favorable drugs due to low cost, low molecular weight, and easy availability. As demonstrated, they exhibit significant biological activity against mammalian chitinases which have been implicated in several inflammatory disorders and cancers. Therefore, their use as immune-modulators will surely provide new therapeutic approaches.

ANTI-INFLAMMATORY EFFECTS OF CAFFEINE, THEOPHYLLINE, AND PENTOXIFYLLINE

The major anti-inflammatory effects of caffeine, pentoxifylline, and theophylline result from 2 main mechanisms; the non-selective inhibition of phosphodiesterases (PDEs) and as a non-selective adenosine receptor antagonist. Through the inhibition of PDEs, a rise in intracellular cyclic adenosine mono-phosphate, activation of protein kinase A, inhibition of tumor necrosis factor alpha (TNFα) and leukotriene synthesis, and reductions in in-

Table 2 Major biological effects of methylxanthine derivatives

Compound	Molecular weight (g/mol)	Biological effects	Side effects
Caffeine	194.19	Increases alertness, slightly increases metabolic rate ^[80] , increases blood pressure, is a diuretic, improves sports performance ^[81]	Caffeine dependency, restlessness, insomnia, and anxiety at high levels (250-500 mg daily ^[82]) In extreme amounts (> 600 mg daily chronically): Gastrointestinal (GI) disturbance ¹ , irregular/rapid heartbeat, mania, depression, and psychosis ^[83]
Pentoxifylline	278.31	Improves blood circulation through peripheral blood vessels, prevents nausea/ altitude sickness, improves red blood cell deformability (<i>i.e.</i> , sickle cell anemia), reduces blood viscosity, reduces formations of platelet aggregation/thrombus ^[84]	Irregular heartbeat, chest pain, dizziness, edema in extremities Acute toxicity in rats determined at 1772 mg/kg ^[85]
Theophylline	180.164	Relaxes bronchial smooth muscle, increases heart contractility, rate, and efficiency, increases blood pressure, increases renal circulation, stimulates respiratory center of CNS, treatment for COPD, asthma, infant apnea ^[86,87]	Interactions with many drug (cimetidine and phenytoin), and causes nausea, arrhythmias, insomnia, irritability, dizziness, seizures and tachyarrhythmias at toxic concentrations (> 20 mg/mL) ^[86]

¹GI disturbance is marked by nausea, vomiting, abdominal pain, diarrhea, bowel incontinence, and anorexia. COPD: Chronic obstructive pulmonary disease; CNS: Central nervous system.

flammation and innate immunity are observed^[43-46]. Deree *et al.*^[43] reported that pentoxifylline successfully reduced TNF α production after human mononuclear cells were stimulated with lipopolysaccharide. A similar result was found in peripheral blood monocytes and alveolar macrophages from sarcoidosis patients, in which pentoxifylline also inhibited the spontaneous TNF α production associated with this disease^[44]. Therefore these compounds may be useful in reducing LPS-induced inflammation and as a treatment for sarcoidosis. Methylxanthine derivatives demonstrate non-selective inhibition of all PDEs by competitive inhibition and therefore they likely bind to the active site of PDEs, however their exact molecular mechanism of inhibition is still uncertain. Caffeine and theophylline were shown to inhibit several PDE isozymes to a similar extent, and the two compounds showed an almost equal affinity for each of the PDE isozymes^[47].

Through the inhibition of leukotrienes, which are known as pro-inflammatory mediators involved in asthma and bronchoconstriction and which play pivotal roles in innate immunity, asthma symptoms are relieved and inflammation is reduced^[46]. Leukotrienes enhance inflammation by increasing leukocyte infiltration, phagocyte microbial ingestion, and generation of pro-inflammatory cytokines including IL-5, TNF α , and macrophage inflammatory protein-1 β ^[48]. It was proven that theophylline effectively reduced leukotriene synthesis and reduced chemotaxis of complement 5a- and platelet-activating factor-stimulated human eosinophils obtained from normal and atopic donors^[49].

As a non-selective receptor antagonist for adenosine, methylxanthine derivatives, most notably, caffeine, are well known as wakefulness aids, as adenosine is a known inducer of sleep. Caffeine, theophylline, and pentoxifylline non-selectively affect several adenosine receptors, including A₁, A_{2A}, A_{2B}, and A₃. A₁ receptor is found ubiquitously throughout the body and studies demonstrated inhibition of this receptor with the novel compound L-97-1 [3-[2-(4-aminophenyl)-ethyl]-8-benzyl-7-{2-eth-

yl-(2-hydroxy-ethyl)-amino]-ethyl}-1-propyl-3,7-dihydro-purine-2,6-dione] reduce histamine and/or adenosine-induced hyperresponsiveness and early and late allergic responses in a rabbit model of house dust mite-induced allergic reactions^[50]. The A_{2A} receptor is similar to the A₁ receptor in that it is found throughout the body. Mice deficient in A_{2A} receptor had significantly higher levels of the pro-inflammatory cytokines TNF α , IL-12 p40, and IL-6 in an LPS-induced model of septic shock as compared to wild-type mice. Therefore, it is suggested that this receptor plays a pivotal role in controlling excess inflammation/tissue damage^[51]. In a mouse model of allergic asthma induced by AMP or 5-N-ethylcarboxamidoadenosine, antagonizing the A_{2B} receptor with the compound CVT-6883 resulted in decreased cellular infiltration in bronchoalveolar lavage fluid including eosinophils and lymphocytes and reduced bronchoconstriction. Interestingly, a similar but slightly blunted response was also seen in theophylline treatment at 36 mg/mL aerosolization for 5 min^[52]. Therefore, this receptor is a target for asthma patients and CVT-6883 is currently undergoing clinical trials.

A novel area of investigation of methylxanthine derivatives is the anti-inflammatory effects through the inhibition of mammalian chitinases, including CHI3L1. As previously discussed, methylxanthine derivatives are effective pan-chitinase inhibitors^[10]. CHI3L1 has been shown to play a role in many inflammatory disorders including rheumatoid arthritis, asthma, hepatitis, and IBD^[2,6]. CHI3L1 increases inflammation in human bronchial epithelium by inducing IL-8 and activating the MAPK and nuclear factor- κ B pathways, which are involved in cell survival^[53]. IL-8 inhibition was hypothesized to be an effective treatment for asthma-related inflammation/remodeling. In a model of DSS-induced colitis, caffeine treatment at 2.5 mmol/L was shown to decrease TNF α , INF γ , IL-4 in mesenteric lymph nodes, and IL-17F in mesenteric lymph nodes and colon and increased the anti-inflammatory IL-10 production in spleen, mes-

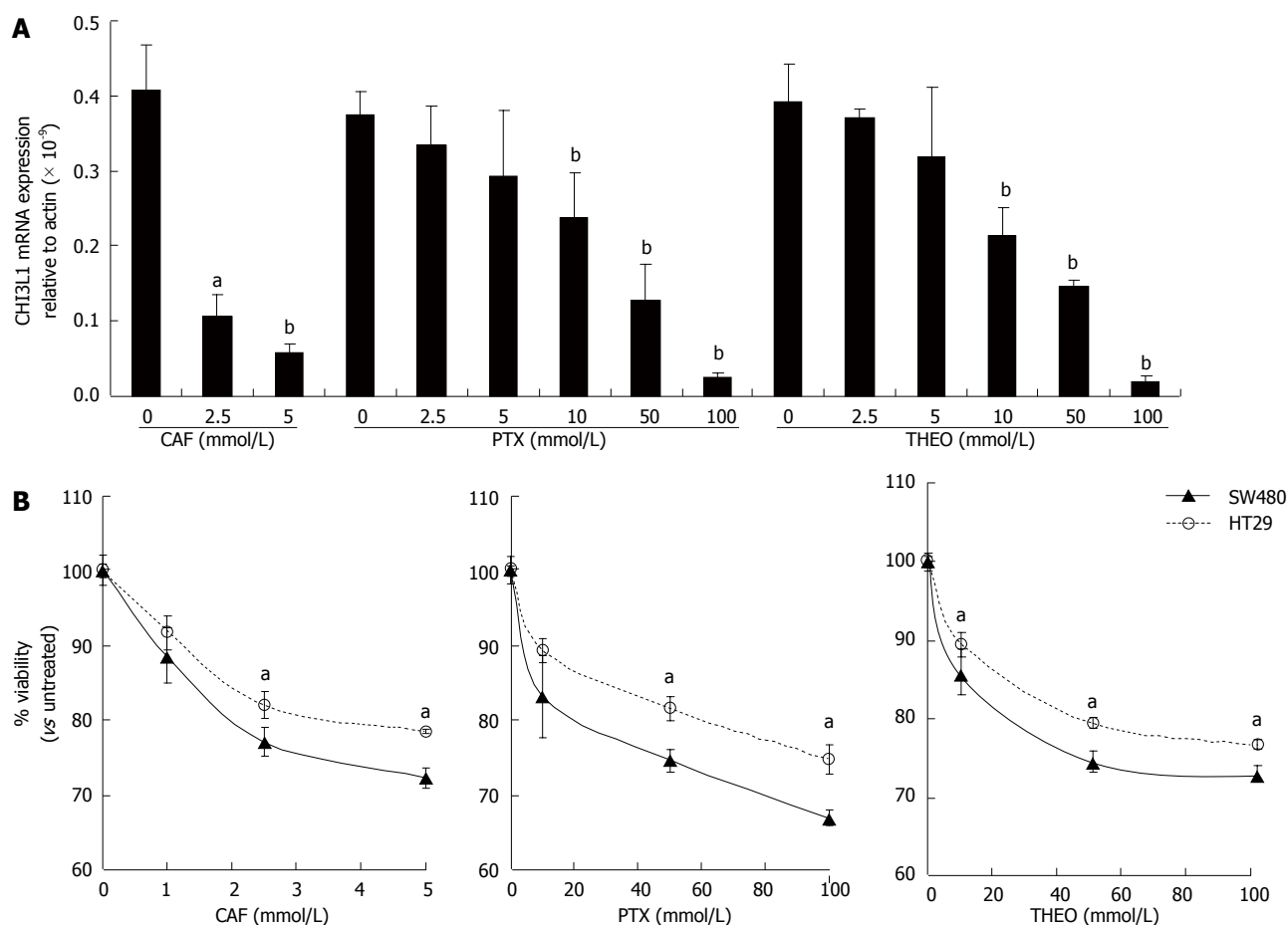


Figure 1 Caffeine, pentoxifylline and theophylline down-regulate chitinase 3-like 1 mRNA expression and reduce cell viability in human colonic epithelial cells. A: SW480 cells were stimulated with caffeine (CAF) at 0, 2.5 or 5 mmol/L or pentoxifylline (PTX) or theophylline (THEO) at 0, 2.5, 5, 10, 50 or 100 mmol/L for 48 h and detected for the chitinase 3-like 1 (CHI3L1) mRNA expression by quantitative-polymerase chain reaction. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal control; B: SW480 and HT29 cells were treated with either CAF (0, 1, 2.5 or 5 mmol/L), PTX or THEO (0, 10, 50 or 100 mmol/L) for 48 h and cell viability were determined using trypan blue exclusion test. CAF, PTX and THEO were purchased from Sigma-Aldrich (St Louis, MO, United States). ^a $P < 0.05$, ^b $P < 0.01$ vs control group.

enteric lymph nodes, and colon^[7,15].

Taken together, methylxanthine derivatives have demonstrated efficacy against the inflammatory disorders, and were shown to reduce inflammation in mice treated with DSS. Therefore, the efficacy of methylxanthine derivatives as potential anti-inflammatory and anti-cancer agents should be further elucidated in other inflammatory conditions and inflammation-associated cancers.

EFFECTS OF METHYLXANTHINE DERIVATIVES ON IBD MOLECULAR PATHWAYS ASSOCIATED WITH CHI3L1

The pathological involvement of CHI3L1 in many diseases, including autoimmune diseases (*e.g.*, IBD, asthma and RA), as well as many forms of solid tumors (*e.g.*, colorectal cancer) are becoming increasingly apparent at this time. The most direct evidence is a significant amount of CHI3L1 induction during the disease state (*e.g.*, IBD and IBD-associated cancer) which activates several important cellular pathways, including AKT and

the β -catenin signaling pathway, thus playing crucial roles in disease pathogenesis^[2,3,6,54]. Characterization of these CHI3L1-mediated pathological pathways can facilitate a better understanding on the molecular mechanisms behind how methylxanthine derivatives can ameliorate diseases through the inhibition of CHI3L1.

In addition to direct protein inhibition of the family 18 chitinases, as determined by X-ray crystallography, *in vitro* methylxanthine treatment in SW480 colonic epithelial cells (CECs), a human colon cancer cell line, directly results in a down-regulation of CHI3L1 mRNA levels (Figure 1A)^[15]. The effective dose of caffeine that is optimal for achieving such down-regulation ranges from 2.5 to 5 mmol/L (Figure 1A). Nevertheless, it was previously shown that 1.0 mmol/L caffeine treatment is sufficient to cause a down-regulation of CHI3L1 in SW480 CECs^[15]. Caffeine treatment also results in the down-regulation of other mammalian chitinases including AMCase, but not chitinase 1^[15]. The effective dose of pentoxifylline and theophylline to down-regulate CHI3L1 in SW480 cells ranges from 10 to 100 mmol/L, whereby any concentration below that did not show any effects on CHI3L1

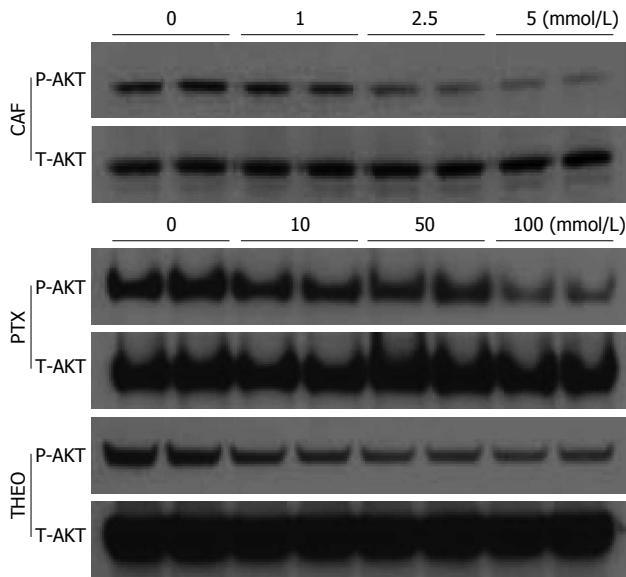


Figure 2 Caffeine, pentoxifylline and theophylline suppress protein kinase B signaling pathway activation in mouse colonic epithelial cells. CMT93 mouse colonic epithelial cells were stimulated with caffeine (CAF) (0, 1, 2.5 or 5 mmol/L), pentoxifylline (PTX) or theophylline (THEO) (0, 10, 50 or 100 mmol/L) for 48 h. Twenty five micro grams of total protein were resolved using SDS-polyacrylamide gel electrophoresis and analyzed by Western blot using anti-phospho/total protein kinase B (AKT) Abs purchased from cell signaling technology (Danvers, MA, United States).

mRNA expression (Figure 1A). Since rabbit anti-CHI3L1 antibody administration to mice has been shown to have an ameliorating effect in acute DSS-induced colitis development, the direct down-regulation of CHI3L1 using methylxanthine derivatives also achieves a similar therapeutic effect in IBD *in vivo*^[2,15].

Furthermore, methylxanthine derivative treatment also reduces colon cancer cell viability in a CHI3L1 expression dependent manner. *In vitro* treatment with caffeine (1-5 mmol/L), pentoxifylline (10-100 mmol/L) or theophylline (10-100 mmol/L) in SW480 cells that express high endogenous CHI3L1 greatly reduces the viability of cells (Figure 1B). However, methylxanthine derivative treatment in HT29 CECs, a human colon cancer cell line that does not express endogenous CHI3L1, has minimal effect on cell viability, indicating a direct involvement of cell survival that is mediated by CHI3L1 expression, at least in part. This has important implications in carcinogenesis since many solid tumors, including colorectal cancer and breast cancer, exhibit exaggerated expression of CHI3L1^[6]. Mechanistically, CHI3L1 directly contributes to tumorigenesis by exerting excessive cell proliferation and angiogenesis^[54,55]. Thus, methylxanthine derivative treatment provides a proof-of-concept in controlling carcinogenic changes and progression by regulating cell viability *via* targeting ectopic CHI3L1 expression and function.

Several studies have demonstrated that AKT signaling is up-regulated in the IEC crypts of chronic UC and CD patients, as well as in a murine DSS-induced colitis model^[56,57]. In contrast, colitis patients that had undergone

5-aminosalicylic acid (5-ASA) treatment showed reduced AKT-phosphorylation in inflamed tissues, suggesting a direct relationship between AKT signal activation and disease severity^[58]. A progressive increase in the densities of phosphorylated AKT in tumor-associated macrophages was observed in normal, colitic and dysplastic to cancer patient specimens^[59]. This expression pattern is in parallel to that of colonic CHI3L1 levels, which showed almost undetectable expression in normal colon, but is induced during colitis that further up-regulates during colitis-associated cancer development^[2,54]. CHI3L1 can directly activate colonic AKT signaling, specifically *via* the 325th-339th amino acid residues within the chitin-binding motif^[3]. This enhanced up-regulation of CHI3L1 during colitis-associated cancer development may provide a plausible explanation for the exaggerated enhancement of AKT phosphorylation. In the context of tumorigenesis, activation of this AKT signaling in the colon induces proliferative signals in IECs that is critical for G1 cell cycle progression^[60]. Thus such constitutive activation of AKT, at least in part mediated by CHI3L1, might result in the uncontrolled cell proliferation. With this in mind, reducing AKT activation by targeting CHI3L1 using methylxanthines seemed to be a possible therapeutic strategy for inflammatory disorders. The combinatory effect of CHI3L1 protein inhibition, as well as direct down-regulation of CHI3L1 mRNA expression by caffeine, pentoxifylline and theophylline, was shown to significantly reduce AKT phosphorylation (Figures 1 and 2 and data not shown)^[15]. The minimum dose of caffeine to achieve a reduction in AKT activation appears to be 2.5 mmol/L, whereas the effective dose of pentoxifylline and theophylline ranges from 10-100 mmol/L.

Another important signaling pathway in the colon that can be activated by CHI3L1 is the β -catenin pathway. Stimulation of SW480 CECs using low dose of CHI3L1 results in an apparent β -catenin nuclear translocation^[6]. In contrast to SW480 cells stimulated with CHI3L1 (80 ng/mL) that predominantly showed a nuclear localization of β -catenin, cells that were stimulated with CHI3L1 and concurrently treated with caffeine (5 mmol/L), but less significant with pentoxifylline (100 mmol/L) or theophylline (100 mmol/L), showed cytoplasmic β -catenin localization (Figure 3). Canonical activation of β -catenin requires the binding of the Wntless (Wnt) ligand onto the Frizzled receptor that subsequently stabilizes cytoplasmic β -catenin by destroying a protein complex (AXIN, GSK3 β and APC) which usually cause the proteolysis of β -catenin under steady-state. This then facilitates the free β -catenin to migrate into the nucleus and subsequent activates transcription of target genes including *c-Myc* and *cyclin D1* (Figure 4). In a cohort study, high activation of β -catenin was found in 100%, 55% and 50% in IBD with colitis-associated cancer, IBD with dysplastic and IBD with remote dysplasia patients, respectively^[61]. Recently, Lee *et al*^[62] identified phosphatidylinositol 3-kinase (PI3K)/AKT signaling as the crucial factor mediating β -catenin during mucosal

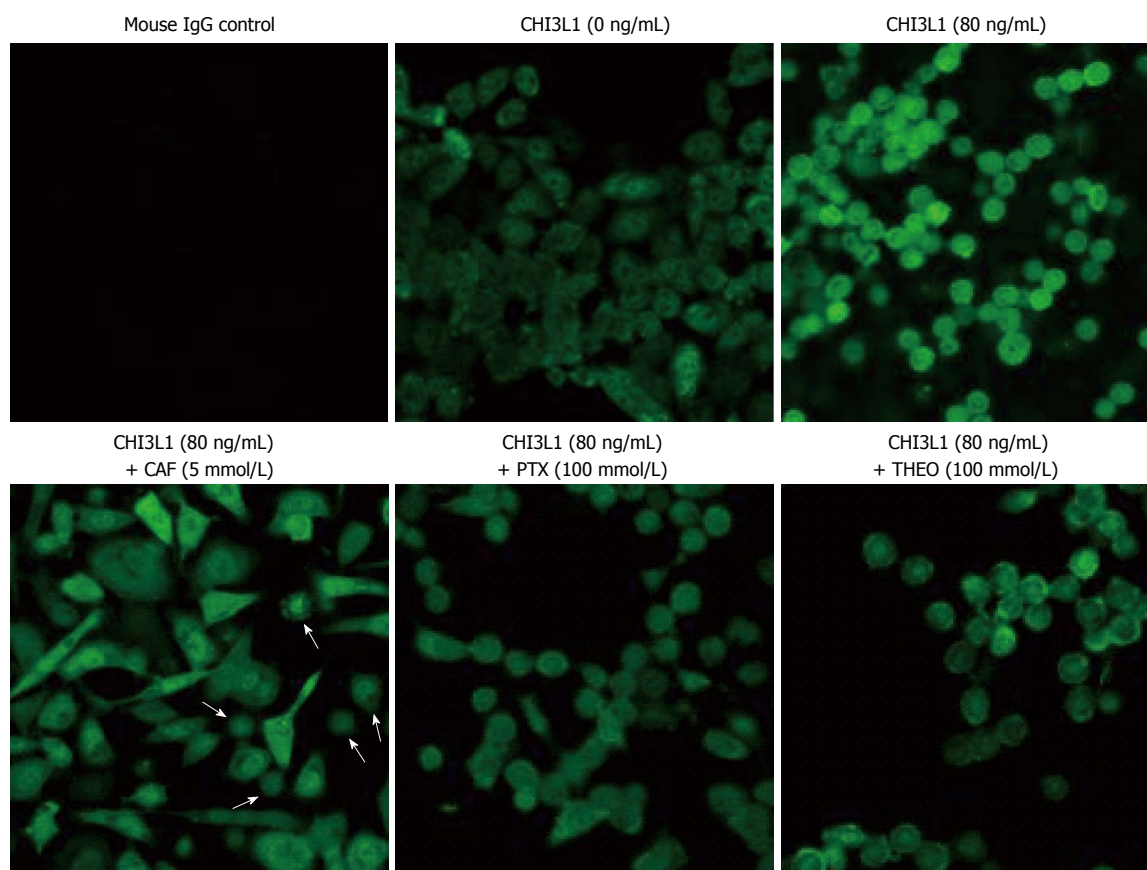


Figure 3 Caffeine, pentoxifylline and theophylline inhibit β -catenin nuclear translocation with different degrees. SW480 colonic epithelial cells were cultured on lab-tec chamber slide. After reached to 90% confluency, the cells were stimulated with or without purified human chitinase 3-like 1 (CHI3L1) (80 ng/mL) in combination with caffeine (CAF) (5 mmol/L), pentoxifylline (PTX) (100 mmol/L) or theophylline (THEO) (100 mmol/L) for 24 h. Human CHI3L1 protein was purchased from Quidel (San Diego, CA). β -catenin was then detected using mouse anti-human β -catenin monoclonal primary Ab (BD Biosciences, CA) and FITC-horse anti-mouse Immunoglobulin G (Vector Labs, Burlingame, CA) and analyzed by confocal microscope (magnification, objective 40 \times). White arrows show the limited numbers of completely nuclear translocated β -catenin positive cells after caffeine treatment.

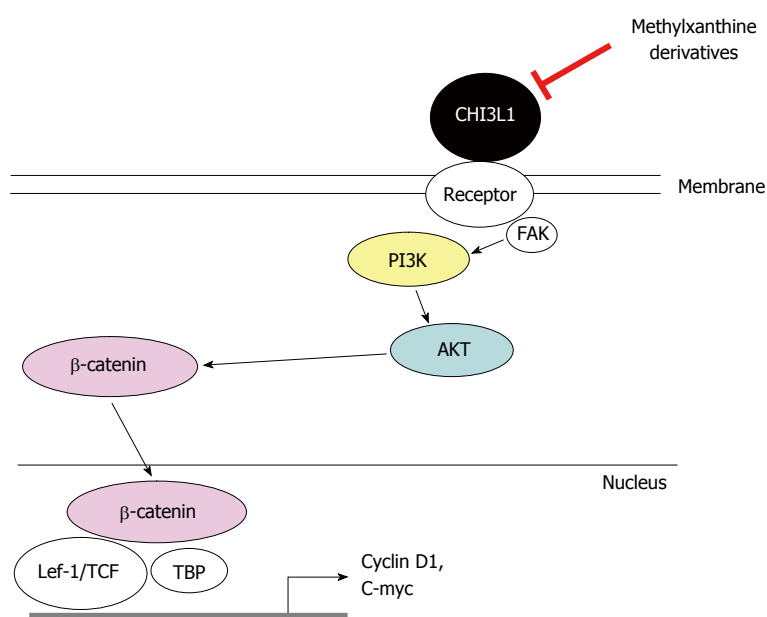


Figure 4 Schematic representation of chitinase 3-like 1-associated β -catenin activation signaling pathway, which is inhibited by methylxanthine derivatives. Binding of extracellular chitinase 3-like 1 (CHI3L1) to a putative receptor on plasma membrane activates the intracellular phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway, which leads to β -catenin activation by translocating this protein from cytoplasm into nucleus. Methylxanthine derivatives, including caffeine, pentoxifylline and theophylline, directly down-regulate CHI3L1 mRNA expression and inhibit CHI3L1 protein functions, leading to reduced CHI3L1-associated AKT activation and prevent down-stream β -catenin nuclear translocation with different degrees of efficacy.

inflammation. They reported that IEC-specific PI3K conditional knockout mice showed reduced AKT and β -catenin signaling in the intestinal stem and progenitor

cells and limits the extent of crypt epithelial proliferation. Inhibiting PI3K in IL-10 knockout mice, which develop spontaneous colitis, also impairs colitis-induced

epithelial AKT and β -catenin activation. Furthermore, a report by Fukumoto *et al.*^[63] also supports that viewpoint that AKT increases β -catenin activity by interfering with the AXIN/GSK3 β complex. Chronic UC patients that have undergone 5-ASA treatment also show reduced AKT-mediated β -catenin phosphorylation in the middle and upper crypts in colon. This observation was recapitulated in 5-ASA treated IL-10 knockout colitic mice^[64]. However, whether CHI3L1 mediated β -catenin activation is exerted directly through the Wnt or AKT pathway, or both but at different temporal time points or cell specific manner, remains to be investigated.

Currently, only a few receptors are known to bind to CHI3L1. Recently, He *et al.*^[65] identified that CHI3L1 binds to the interleukin-13 receptor $\alpha 2$ (IL-13R $\alpha 2$) and activates both AKT and β -catenin signaling in the IL-13R $\alpha 2$ dependent pathway. Therefore, exploring the use of methylxanthine derivatives for inhibiting CHI3L1 may block any downstream effects pertaining to AKT and/or β -catenin signaling and will provide direct mechanistic insights (Figure 4).

POTENTIALLY THERAPEUTIC/ PROPHYLACTIC EFFECTS OF METHYLXANTHINE-DERIVATIVES IN IBD IN VIVO

Recently, our group performed in depth analysis of the role of caffeine treatment in a DSS-induced colitis model in mice^[15]. Our *in vivo* analysis involved prophylactic-, simultaneous-, and post-treatment of mice with caffeine at 2.5 mmol/L in this animal model of intestinal epithelial damage. After initial caffeine treatment for 7 d, we challenged the mice with DSS in the drinking water for 5 d, and then returned to normal drinking water for 7 d before sacrificing. Mice which received the caffeine treatment protocol showed significantly improved symptoms as demonstrated by less percentage bodyweight loss and improved clinical scores. Colons of the mice were isolated, and it was shown that CHI3L1 and AMCase expressions were both significantly decreased after caffeine treatment. In contrast, chitinase 1 expression remained stable after the treatment. Colonic sections were also analyzed for histological changes. Mice in the caffeine-treated group demonstrated improved histological scores, with markedly decreased accumulation of immune cells, including F4/80⁺, CD4⁺, or CD11b⁺ cells. Interestingly, bacterial colony forming units from homogenized mouse spleens, mesenteric lymph nodes, liver, cecum and colon were all significantly reduced after 2.5 mmol/L caffeine treatment. In addition, as we described in the previous section, the levels of several pro-inflammatory cytokines were significantly decreased in spleen, mesenteric lymph nodes, and colon, with an increase in the anti-inflammatory cytokine IL-10 in tissues. A major factor in IBD development is host-microbial interactions including adhesion/invasion of bacteria into

the CECs and lamina propria. Caffeine treatment at both 2.5 and 5 mmol/L effectively prevented AIEC from invading into SW480 CECs, as well as in mouse-derived peritoneal macrophages. This result provides a possible explanation on therapeutic potential of caffeine in IBD through the prevention of CHI3L1-mediated bacterial adhesion/invasion.

In vivo testing using pentoxifylline to study the effects on IBD was also reported. Peterson *et al.*^[66] demonstrated that intra-rectal administration of pentoxifylline or 1-(5-hydroxyhexyl)-3,7-dimethylxanthine (so called metabolite-1 or M-1) in a murine 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model, which showed an attenuation of colonic inflammation and intestinal fibrosis. M-1 is a chiral molecule derived from pentoxifylline by the reduction of a single ketone group to a corresponding hydroxy group. They reported that 64 mg/kg of pentoxifylline or M-1 is an ideal therapeutic dose in mice, whereas mice treated with 32 mg/kg showed varied effects in disease-associated phenotype. Another study also showed similar amelioration by pentoxifylline in TNBS-colitis in rats^[67]. Interestingly, Murthy *et al.*^[68] showed that combining pentoxifylline with anti-TNF α antibody in DSS-induced colitic mice can reduce the side effects that is associated with anti-TNF α antibody treatment alone. *Ex vivo* studies also showed that peripheral mononuclear cells, which are obtained from the inflamed mucosa of CD and UC patients, reduce TNF α secretion by 50% in the presence of pentoxifylline (up to 100 μ g/mL) for 24 h^[69].

FUTURE OPTIMIZATION OF METHYLXANTHINE DERIVATIVES FOR IMPROVED SPECIFICITY AND EFFICACY INHIBITION OF FAMILY 18 CHITINASES

The early discovery of the allosamidin-derived from *Streptomyces* as a chitinase inhibitor has opened up opportunities to test the inhibitory effect on controlling chitinase-associated diseases^[70]. For instance, as demonstrated in a study on AMCase-associated asthmatic Th2 inflammation mouse model, allosamidin, or anti-AMCase antibody, both independently can reduce bronchoalveolar lavage inflammation^[71]. However, the concern over using allosamidin is its broad range of activity against all family 18 chitinases and less than ideal chemical properties (*e.g.*, high molecular weight and poor ligand efficiency)^[72]. In addition, allosamidin has a stronger inhibitory effect on chitinase 1 than AMCase and therefore, since chitinase 1 is highly regarded as a molecule involved in host-defence system against a chitin-containing pathogen rather than a driver molecule involved in allergic inflammation, there is a need to identify or develop other chitinase inhibitor with higher specificity^[73-75]. The discovery of the methylxanthine derivative inhibitory effects on family 18 chitinases appears to represent a promising alternative for its more suitable chemical properties and advantages as

described above sections. Yet, being a pan-chitinase inhibitor, it still faces a similar challenge in target specificity. Therefore, the next step is to optimize both specificity and efficacy of these methylxanthine derivatives.

In order to improve the inhibitory properties of the methylxanthine derivatives, Schüttelkopf *et al.*^[76] has developed a virtual algorithm method to create better family 18 chitinases. The algorithm, named as LIGTOR, basically fixed the methylxanthine substructure while performing torsional evaluation of the substitution based on previous published chitinase-pentoxifylline complex. Upon identification of the most desirable chemical features using this algorithm, the group then subsequently developed a low micromolar chitinase inhibitor that is composed of a two linked caffeine moieties that binds in the active site of the target extensively in a manner that was not previously reported. This di-caffeine compound, subsequently named as bisdionin B, showed the desired drug-like structure, as demonstrated by X-ray crystal structure analysis, and provides a general scaffold for future development/optimization of the family 18 chitinase inhibitors.

Another major concern in drug design is target specificity. As a pan-chitinase inhibitor, one of the major drawbacks of methylxanthine derivatives is the discrimination between the different chitinases (*e.g.*, CHI3L1, AMCase and chitinase 1). To address this issue, Sutherland *et al.*^[77], utilized the LIGTOR algorithm derived di-caffeine scaffold and modified the caffeine linker length and subsequently analysed it against the AMCase crystal structure. They then developed a derivative of the di-caffeine scaffold, termed as bisdionin F, that showed a high selectivity for human AMCase up to 20-fold over chitinase 1. The exact orientation/coordinates were confirmed by crystal structure of the human AMCase-bisdionin F complex. The group further validated the efficacy of bisdionin F in a murine model of allergic inflammation. All these suggest that further improvements can be made to develop a molecule with improved inhibitory efficacy and higher specificity against the targeted molecule of the chitinase 18 family.

CONCLUSION

CHI3L1 is an important inducible molecule on IECs and actively participates in the pathogenesis of chronic inflammation and inflammation-associated malignant transformation of epithelial cells. Methylxanthine derivatives, including caffeine, theophylline and pentoxifylline, can potentially suppress inflammation *via* CHI3L1 inhibition. The result in this study may provide the conceptual framework for a new class of therapeutic agents, which will effectively prevent chronic inflammatory diseases with minimal side effects.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

When combination therapy isn't working: Emerging therapies for the management of inflammatory bowel disease

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Abstract

Although antagonists of tumor necrosis factor have resulted in major therapeutic benefits in inflammatory bowel disease, the magnitude and durability of response are variable. Similar to previously available drugs such as 5-aminosalicylates and immunomodulators, the therapeutic effect is not universal leaving many people searching for options. The development of newer agents has benefited from advances in the understanding of the pathophysiology of the disease. Uncontrolled activation of the acquired immune system has an important role, and lymphocytes, cytokines, and adhesion molecules are broadly targeted for therapeutic intervention. There is increasing evidence of an important role of the innate immune system and the intestinal epithelium, and the therapeutic paradigm is also shifting from immunosuppression to the reinforcement of the intestinal barrier, and modification of the disease process. In this review, we explore the limitation of current therapy as well as mechanisms of actions of new drugs and the efficacy and adverse events from data from clinical trials.

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Key words: Inflammatory bowel disease; Emerging

therapies; Vedolizumab; Ustekinumab; Tofacitinib

Core tip: In this paper we critically review recently published literature about these novel therapies, which have been the results of extensive research identifying molecular targets. Several agents have been tested and show promising data, but we focus on vedolizumab, a monoclonal antibody against the $\alpha 4\beta 7$ integrin on lymphocytes, ustekinumab, a monoclonal antibody against the p40 subunit of interleukin-12 and interleukin-23, and tofacitinib, an orally administered small molecule targeting Janus-activated kinase. These three agents are most likely to find their way soon to the market and offer significant therapeutic advantages for the management of Crohn's disease and ulcerative colitis.

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INTRODUCTION

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are thought to be the result of an overly aggressive immune response toward an environmental trigger within a genetically susceptible host. Recent work has elucidated the host-microbe interaction identifying the complex interplay between genetic susceptibility, environmental factors, and the intestinal microbiome^[1]. This has led to a deeper understanding of the immunologic pathways leading to IBD and, most importantly, to the development of targeted therapies. For CD and UC not responding to 5-aminosalicylates (5-ASA), immunosuppression with corticosteroids, aza-

thioprine, and anti-tumour necrosis factor (anti-TNF) antibodies have been the mainstay of treatment^[2]. Even the most potent combination of immunomodulator and anti-TNF therapy in combination in recently diagnosed CD only achieves steroid free remission in 57% of patients^[3]. Given the persistent and sizable population of patients who are not served with current therapy, there has been great interest in new pathways of inflammation that would be amenable to pharmacologic intervention. This paper focuses on the most promising pathways and medications that appear closest to clinical availability.

LIMITATIONS OF CONVENTIONAL THERAPY

Corticosteroids

The significant benefit of corticosteroid therapy in IBD was established in the 1950s and 1960s for UC and later in the 1970s and 1980s for CD^[4]. In general, corticosteroids centrally suppress nuclear factor (NF)- κ B activation, which is the primary transcription factor mediating inflammatory response in both the innate and adaptive immune systems. In active IBD, corticosteroids are still a viable first-line treatment, but patients and clinicians have to be aware of significant short- and long-term side effects. Opportunistic infections, steroid-induced psychosis, steroid dependence, diabetes, and osteoporosis are among the most common side effects^[5]. Clinicians should also be aware of the variability of clinical response to these agents. Corticosteroids are unlikely to induce mucosal healing or maintain clinical remission, so its use is limited to induction of remission^[6].

Thiopurines

Thiopurines, also referred to as immunomodulators, are derivatives of thioguanine and act as purine antimetabolites. Following metabolization into 6-thioguanine nucleotides, immunosuppression is in part the result of incorporation into the DNA/RNA of rapidly dividing inflammatory cell lines^[7]. This induces effector T cell apoptosis by suppression of the Rac1 and Vav-Rac1 signaling pathways and decreases NF- κ B activation, which leads to a decrease in pro-inflammatory cytokine secretion. Up to one third of patients with IBD are intolerant to thiopurines and a further 10% are unresponsive to them^[7]. In the majority of patients who do respond, the benefits of thiopurines take 3-6 mo to appear^[8]. Significant risks of thiopurines include lymphoproliferative disease (non-Hodgkin's lymphoma), as high as 4-5 fold compared with unexposed IBD patients and further increased when used in combination with anti-TNF^[9]. Other side effects include early hypersensitivity reactions such as fever and pancreatitis, bone marrow suppression and hepatotoxicity requiring frequent lab monitoring during treatment^[10]. Finally, one in 300 patients harbor a homozygous *TPMT* mutation and use of these agents is generally avoided^[11].

Methotrexate

For patients with CD unresponsive to thiopurines, metho-

trexate has been the alternative. In a recent study from 2011, Kozarek *et al*^[12] showed that in patients who had failed azathioprine treatment, methotrexate was effective in maintaining clinical benefits in 63% of patients at 1 year. And in this group, 26% required side effects sufficient enough to discontinue therapy, and most of these adverse events occurred in the first 6 mo. This study shows that methotrexate is well tolerated in the long term and is a viable option in patients who cannot receive thiopurines. However, methotrexate has lower mucosal healing rates compared to biologics and azathioprine^[12].

Methotrexate is an antimetabolite and acts specifically during DNA and RNA synthesis and therefore its effect is mainly seen on rapidly dividing cells (such as gastrointestinal and oral mucosa, and effector T-cells). This mechanism of action explains the most frequently observed side effects of myelosuppression and liver. Furthermore, its absolute contraindication in pregnancy makes this drug less desirable to use in reproductive aged women^[13]. Unlike, thiopurines methotrexate is not effective in inducing or maintaining remission in UC^[14]. In addition, a significant percentage of prescribing gastroenterologists were unfamiliar and uncomfortable with its use in the management of CD, further limiting its use in the United States^[15].

Biologic therapy

Anti-TNF- α inhibitor therapy: TNF- α is a key pro-inflammatory cytokine involved in the systemic inflammatory cascade and is a member of a group of cytokines that stimulate the acute phase reaction. In response to lipopolysaccharide and other bacterial products presented by antigen-presenting cells, activated intestinal T cells, macrophages, produce TNF- α and natural killer cells. In IBD, the number of TNF- α secreting T cells in the lamina propria is increased and specific agents directed at these T cells were developed^[16].

The United States Food and Drug Administration (FDA) approved the first anti-TNF agent for CD, infliximab, in 1998. This was followed by additional in category drugs, adalimumab in 2007, which had the benefit of subcutaneous administration, and certolizumab in 2008.

While various trials have confirmed the significant clinical benefit of anti-TNF- α therapy, it is apparent that there are major limitations in the use of these agents, ranging from cost-effectiveness issues to morbidity and mortality, and the side effect profile is significant^[17]. Data from large treatment registries suggest that patients receiving both anti-TNF- α agents and other immunosuppressants such as azathioprine and corticosteroids are at a higher risk of developing opportunistic infections and lymphoma^[9]. In addition, approximately 10% per year of patients lose response to this therapy, often but due to the development of anti-drug antibodies^[18,19].

Therapies targeting other cytokine pathways

Natalizumab: Natalizumab, a human anti- α 4 integrin antibody, has been studied in CD^[20] and a recent meta-analysis concluded that it was superior to placebo in

controlling symptoms and inducing mucosal healing^[16]. However, its adoption was limited by the discovery of the development of progressive multifocal leukoencephalopathy in 1:1000 patients^[21]. After initially being removed from the market, it's currently available only through enrollment in a specialized FDA risk minimization program known as TOUCH (<https://www.touchprogram.com/TTP>) that details the risk of progressive multifocal leukoencephalopathy (PML).

New therapeutic goals: Mucosal healing and deep remission: In recent years, mucosal healing has emerged as a major therapeutic goal in IBD. However, the definition of mucosal healing varies across studies, and there is no validated definition of mucosal healing or endoscopic remission in IBD^[22]. Current therapeutic goals are to induce remission both clinically, biochemically and endoscopically^[23].

“Deep remission” in CD is defined as a combination of endoscopic and clinical indicators of disease activity, a CD activity index (CDAI) below 150 together with complete mucosal healing. Deep remission has been associated with better long term disease-specific quality of life, fewer flares and Crohn's related hospitalizations, greater work productivity, and less activity impairment compared to mucosal healing alone^[24]. In UC, there is no proposed definition of deep remission.

Another area of consensus regarding potential therapy is prevention of bowel damage in CD by earlier introduction potent of therapy. In a population-based cohort study from Olmsted County, United States, among patients with CD diagnosed from 1970 to 2004, the cumulative risk of developing complications (STRUCTURING should be stricturing or penetrating disease) was 34% at 5 years and 51% at 20 years after diagnosis^[25]. These observations highlight the importance of creating tools that are able to measure cumulative bowel damage in CD. The development of an appropriate index is underway and has been named the CD digestive damage score (the Lemann score)^[26]. It addresses damage location, severity, extent, progression and reversibility, as measured by diagnostic imaging modalities and the history of surgical resection. The Lemann score may be used to assess the effect of various pharmacological therapies, function as a clinical trial endpoint and allow better identification of high-risk patients in regard to identification or progression of bowel damage.

The concept of early treatment to avoid later complications and the need for surgical intervention in CD is gaining momentum, and these new scoring systems might be able to help to identify patients at risk and help caregivers determine the timing for introduction of disease modifying agents. In a population-based study from Cardiff, United Kingdom, Ramadas *et al*^[27] reported that early thiopurine use (within the first year of diagnosis) was associated with lower rates of surgery. Subgroup analysis from placebo-controlled trials with anti-TNF- α agents have also suggested that patients with early CD may ex-

perience greater efficacy than patients with established disease^[28].

Complicated CD can be defined as the presence of bowel damage (stricture, abscess and/or fistula) and/or the requirement for surgery. The clinical factors associated with complex CD include: ileal disease, upper gastrointestinal involvement, smoking, complicated behavior (stricturing or penetrating), young age at diagnosis, perianal disease and some genetic factors such as nucleotide oligomerization domain 2 (*NOD2*), interleukin 10 (*IL10*) or *IL-10R* mutations^[29]. The most widely studied genetic marker in CD is the presence of variants of the *NOD2* gene and its association with a more complex disease course and requirement for surgeries^[30]. The IBD CHIP project, a new DNA array-based diagnostic system which uses a DNA array to detect IBD mutation to predict the clinical evolution for CD, found that the *NOD2* gene was the most important genetic association, was an independent predictive factor for need for surgery, and the strongest factor associated with a complicated disease course. However, a more recent study has reported that associations with gene variants are not accurate in predicting the course of CD. Currently, serological and genetic markers are not routinely used in clinical practice, as data are variable in the predictive value of these markers^[31-33].

Aggressive UC was recently defined as disease that is associated with a high relapse rate, the need for surgery, the development of colon cancer and/or the presence of extra-intestinal manifestations^[34], though this marks a very heterogeneous group of patients. Clinical risk factors include extensive colitis and a young age at diagnosis. As in trials for rheumatoid arthritis, new emphasis is being placed on disease modifying drugs which limit mucosal damage. This marker of therapy may be used in upcoming disease-modification trials as well as in our clinical practice to promote early intervention with disease modifying agents in patients having such factors^[35].

Emerging concepts in the management of IBD patients are tight monitoring and accelerated step up approaches^[36]. These require endoscopy, imaging (magnetic resonance imaging) and colonoscopy, C reactive protein, and/or fecal markers at 3-6 mo after introduction of disease-modifying agents (thiopurines and anti-TNF- α) to identify objective signs of inflammation, and escalation of treatment. Further clinical trials are needed to evaluate the value of this strategy.

In CD patients with mild disease, corticosteroids are appropriate on an as needed basis, and in patients with moderate active disease without poor prognostic indicators, steroids and thiopurines still remain the standard first-line therapy. Anti-TNF- α therapy should be considered as first-line therapy in patients with CD with bowel damage (stricture/fistula/abscess) and/or poor prognostic factors, severe disease, or complex perianal disease^[33,36].

In UC, a proposed treatment algorithm for accelerated step-up therapy after “5-ASA failure” indicates that steroids and azathioprine should be considered. If

in case of is steroid-dependent disease and persistent signs of inflammation, then long term maintenance therapy with thiopurines or anti-TNF- α agents should be considered^[34,37].

Despite therapeutic advances over the past several years and introduction of multiple new anti-TNF agents, including infliximab, adalimumab, certolizumab, golimumab, and natalizumab (CD only), there is still a large subset of patients that do not respond to these drugs or are unable to maintain remission long-term^[38-41]. It is likely that these patients have disease that is driven by other factors. The best management approach toward these patients is unclear, but the development of new non anti-TNF based therapies may be a promising avenue of treatment. Three new agents in the “pipeline” which appear to be promising, and are closest to being commercially available in the United States are ustekinumab, tofacitinib, and vedolizumab. Ustekinumab already has FDA approval for psoriasis, and tofacitinib has FDA approval for rheumatoid arthritis, vedolizumab will soon be evaluated by the FDA for approval as IBD therapy^[35], and is expected to be approved in 2014.

Targeting IL-12/23: IL-12 and IL-23 are inflammatory cytokines produced by antigen-presenting cells, which promote T cell maturation into T-helper (Th)1 and Th17 phenotypes, respectively. The cytokines were identified to be significant to the pathology IBD in genome wide association studies^[42]. IL-12 and IL-23 share a common p40 subunit, and it is known that when IL-12 (p35 + p40) is present, T cells differentiate into Th1 cells producing interferon- γ and TNF. On the other hand, when IL-23 (p19 + p40) is present together with transforming growth factor- β and IL-6, the Th17 subset preferentially develops and produces IL-6, IL-17A, IL-17F, IL-22 and IL-21^[35]. Since both pathways are activated in CD patients, and contribute to tissue damage by the production of inflammatory cytokines, this makes neutralizations of the p40 subunit an attractive therapeutic target. Ustekinumab (Stelara) and briakinumab (Ozespia, previously ABT-874; Abbott, Abbot Park, IL, United States) are monoclonal IgG1 antibodies that target the p40 subunit of the IL-12/IL-23. Although Phase II trials in briakinumab were negative, however ustekinumab showed promising results in CD and is being further evaluated^[43,44].

The efficacy of ustekinumab was initially investigated in a double-blind, cross-over trial with 104 moderate to severe CD patients. In this group, clinical response rates for the patients given ustekinumab and placebo were 53% and 30%, respectively ($P = 0.02$) at weeks 4 and 6, and 49% and 40% ($P = 0.34$) at week 8. Further subgroup analysis showed that patients who received infliximab in the past (neither primary nor secondary non responders) had a significantly greater response to ustekinumab ($P < 0.05$) through week 8. Based on the results of this study it was noted that ustekinumab induced clinical response in patients with moderate to severe disease, especially in those with prior exposure to infliximab. These results

led to further evaluation of ustekinumab in inducing and maintaining remission in patients with CD refractory to anti-TNF agents. During induction, 526 patients were randomly assigned to receive intravenous ustekinumab (at dose 1, 3, or 6 mg/kg of body weight) or placebo at week 0. In the maintenance phase, the 145 patients who had a response to ustekinumab at 6 wk underwent a second randomization to receive subcutaneous ustekinumab (90 mg) or placebo at weeks 8 and 16, the primary endpoint was clinical response at week 6 defined as decrease in CDAI of 100 points from baseline. The proportions of patients who met the primary endpoint were 36.6%, 34.1%, 39.7% for 1, 3 and 6 mg of ustekinumab, respectively *vs* 23.5% for placebo ($P = 0.005$ for 6 mg group). The proportion of patients who achieved clinical remission was not significantly different between any of the groups at week 6. However, maintenance therapy with ustekinumab, as compared with placebo, resulted in significantly increased rates of response (69.4% *vs* 42.5%, $P < 0.001$) and clinical remission (41.7% *vs* 27.4%, $P = 0.03$) at 22 wk. Serious infections occurred in 7 patients (6 receiving ustekinumab) during induction and 11 patients (4 receiving ustekinumab) during maintenance therapy^[43,45] (Table 1).

We await the results of an ongoing phase 3 study. If the data are confirmed, this therapy may become a useful option for patients who have failed anti-TNF therapy.

ANTIADHESION MOLECULES

Drugs targeting adhesion molecules interfere with the migration of leukocyte subsets from the blood to the sites of inflammation^[46]. The first drug in this category to be used in IBD was a monoclonal antibody against the $\alpha 4$ integrin, natalizumab. The blockade of $\alpha 4$ -integrins not only interferes with the $\alpha 4\beta 7$ MadCam1 interaction, associated with IBD, but also with $\alpha 4\beta 1$ vascular cell adhesion molecule-1 interaction which is needed for patrolling effector T cells to contain JC virus and prevent it from infecting the brain. Following these results, therapies targeting $\alpha 4\beta 7$ more specifically for the gut vasculature were developed in order to avoid the risk of PML^[47].

Vedolizumab (formerly called MLN02, Millenium; Takeda) binds specifically to the $\alpha 4\beta 7$ -integrin dimer which is involved in recruitment of lymphocytes to the gut. Two large phase 3 studies under the GEMINI study group, one in UC (GEMINI 1) and one in CD (GEMINI 2) have recently been published and demonstrate a beneficial effect of vedolizumab in induction and maintenance of remission of UC and CD. GEMINI 1 and 2 are randomized, blinded, placebo-controlled multicenter trials to examine the efficacy of vedolizumab for induction and maintenance in moderate to severe UC and CD, respectively.

In the induction trial of the GEMINI 1 study, 374 patients received vedolizumab or placebo at week 0 and 2, and 521 patients received open-label vedolizumab at weeks 0 and 2 with disease evaluation at week 6. Patients

Table 1 Clinical response defined as decrease in Crohn's disease activity index > 100

Therapeutic agent	Disease tested	Response <i>vs</i> placebo	Target patients	Ref.
Ustekinumab	Crohn's disease	Clinical response: 69.4% <i>vs</i> 42.5%, $P < 0.001$ Remission: 41.7% <i>vs</i> 27.4%, $P = 0.03$	Adult patients with moderate to severe Crohn's disease with failure to anti-tumour necrosis factor (TNF) therapy	[43]
Vedolizumab	Crohn's disease	Clinical response: 31.4% <i>vs</i> 25.6%, $P = 0.23$ Remission at week 52: 14.5% <i>vs</i> 6.8%, $P = 0.02$	Adult patients with active Crohn's disease (Crohn's disease activity index, 220-450) with previously documented lack of response to glucocorticoids or immunosuppressive agents or anti-TNF agents	[52,53]
	Ulcerative colitis	Clinical response: 47.1% <i>vs</i> 25.5%, $P < 0.001$ Remission at week 52: 41.8%, 44.8% (8 and 4 wk dosing interval, respectively) <i>vs</i> 15.9%, $P < 0.001$	Adult patients with active ulcerative colitis (Mayo score 6 to 12) with disease at least 15 cm proximal from anal verge, with previously documented unsuccessful treatment with glucocorticoids or immunosuppressive medications or anti-TNF	
Tofacitinib	Ulcerative colitis	Clinical response: 32%, 48%, 61%, 78% [at dose 0.5 mg ($P = 0.39$), 3 mg ($P = 0.55$), 10 mg ($P = 0.10$), and 15 mg ($P < 0.001$)] <i>vs</i> 42% for placebo Clinical remission: 13%, 33%, 48%, 41% [at dose 0.5 mg ($P = 0.76$), 3 mg ($P = 0.01$), 10 mg ($P < 0.001$), and 15 mg ($P < 0.001$)], <i>vs</i> 10% for placebo	Adults patients with moderate to severe ulcerative colitis	[52]

who had a response at week 6 were then randomly assigned to continue receiving vedolizumab every 8 or 4 wk or were switched to placebo for up to 52 wk. The primary outcome was clinical response at week 6 defined as a reduction in the Mayo score of at least 3 points and a decrease of at least 30% from the baseline score. Response rates at week 6 were 47.1% and 25.5% among patients in the vedolizumab group and the placebo group, respectively ($P < 0.001$). At week 52, 41.8% of patients who continued to receive vedolizumab every 8 wk and 44.8% of patients who received vedolizumab every 4 wk were in clinical remission, as compared with 15.9% of patients who switched to placebo ($P < 0.001$ for both groups). The frequency of side effects was similar in both groups and serious adverse events were not more common in the vedolizumab group. All patients in the trial had an eligibility criterion of an unsuccessful previous treatment (lack of response, or unacceptable side effects), with one or more of the following medications: glucocorticoids, immunosuppressive medications (azathioprine or 6-mercaptopurine), or TNF antagonists. Patients were allowed to continue 5-ASA drugs during the study. This study shows that vedolizumab is more effective than placebo as induction and maintenance therapy in UC and more importantly, shows response in patients who had failed previous therapy^[48,49].

In the induction trial for GEMINI 2, 368 patients were randomly assigned to receive vedolizumab or placebo at weeks 0 and 2 and 747 patients received open label vedolizumab at weeks 0 and 2; disease status was assessed at week 6, and the two primary endpoints were clinical remission (CDAI < 150) and CDAI-100 response (> 100 point decrease in CDAI score) at week 6. In the maintenance trial, 461 patients who had had a response to vedolizumab were re-randomized to placebo or vedolizumab every 8 or 4 wk until week 52. At week 6, a total of 14.5% of patients who received vedolizumab were

in clinical remission, compared to 6.8% of patients who received placebo ($P = 0.02$). A total of 31.4% and 25.7% of patients in the treatment *vs* placebo group had a CDAI-100 response ($P = 0.23$). Among the 461 patients who had an initial response, 39.0% and 36.4% of those assigned to vedolizumab every 8 or 4 wk were in clinical remission at week 52, compared to 21.6% of those assigned to placebo ($P < 0.001$ and $P = 0.004$, for the two vedolizumab groups, respectively). Vedolizumab, as compared with placebo, was associated with a higher rate of serious adverse events (24.4% *vs* 15.3%), infections (44.1% *vs* 40.2%), and serious infections (5.5% *vs* 3.0%). Eligible patients for the trial had had no response to or had had an unacceptable side effects from one or more of the following: glucocorticoids, immunosuppressive agents (azathioprine, 6-mercaptopurine, or methotrexate), or TNF antagonists. This study shows that vedolizumab-treated patients with active CD were more likely than patients receiving placebo to have a remission, but not a CDAI-100 response, at week 6 (primary endpoint); and that those patients with an initial clinical response were more likely to be in clinical remission at week 52^[50].

Both these reports are among the largest clinical studies in patients with IBD and combined consist of 2010 patients. Primary and secondary endpoints were met in the GEMINI 1 study (UC), however it seems that vedolizumab was less effective in patients with CD, although maintenance of remission was noted in the treatment group, and that patients with CD had a higher frequency of adverse events with treatment compared to placebo. It is possible that the underlying pathogenesis of the two diseases are different, and the selective blockage of gut-specific $\alpha 1\beta 4$ -mediated leukocyte trafficking is more beneficial in UC, which is confined to the mucosa and the large intestine, compared to CD which might represent a more systemic disorder. More studies need to be done to assess the pharmacodynamics of vedolizumab.

To date, there have been no reported cases of PML. The results of these two recent studies suggest a promising new therapy for IBD.

TARGETING JANUS KINASE RECEPTORS

Besides specific neutralization of specific cytokines with antibodies, control of inflammation can be achieved by interfering with conserved elements associated with cytokine receptors, thus allowing a broader action. The Janus kinase (JAK) family of tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) plays a significant role in signal transduction of hematopoietin receptors type I and II. Tofacitinib (formerly CP-690, 550; Pfizer) is a JAK inhibitor that inhibits all four JAK family kinase members, with a functional cellular specificity for JAK1 and JAK3 over JAK2. Thus it can directly or indirectly modulate signaling for an important subset of proinflammatory cytokines including IL-2, -4, -7, -9, -15 and -21^[51].

In a double-blind, placebo controlled, phase 2 trial, 194 patients with moderately to severely active UC were randomized to receive tofacitinib at a dose of 0.5, 3, 10 or 15 mg or placebo twice daily for 8 wk. Patients enrolled had to have a Mayo score of at least 6 with an endoscopic subscore of 2 or 3, and patients could receive oral mesalamine or oral prednisone at a stable dose of 30 mg or less per day, patients were not allowed to be on concurrent immunosuppressive therapy or therapy with anti-TNF agents^[50]. Approximately 30% of patients had had prior anti-TNF exposure with failure of therapy. The primary outcome was clinical response at 8 wk, and occurred in 32%, 48%, 61% and 78% of patients receiving tofacitinib at a dose of 0.5 mg ($P = 0.39$), 3 mg ($P = 0.55$), 10 mg ($P = 0.10$) and 15 mg ($P < 0.001$), respectively, compared with 42% of patients receiving placebo. Clinical remission, defined as Mayo score < 2 with no subscore > 1 , at 8 wk occurred in 13%, 33%, 48%, and 41% of patients receiving tofacitinib at a dose of 0.5 mg ($P = 0.76$), 3 mg ($P = 0.01$), 10 mg ($P < 0.001$) and 15 mg ($P < 0.001$), respectively, compared with 10% of patients receiving placebo. The most commonly reported adverse events was related to infection, and occurred in 6 patients on treatment with any dose of tofacitinib and 6 patients in the placebo group. There was a dose dependent increase in both high and low density lipoprotein cholesterol at 8 wk with tofacitinib which reversed after discontinuation of the study drug^[52]. Given the broad mechanism of action, opportunistic infections remain a valid concern and more data is required to determine the efficacy and safety of tofacitinib in the treatment of IBD. However, its oral method of administration will surely make this a popular avenue of treatment should it prove effective in larger scale treatment trials (Table 1).

CONCLUSION

Although IBD therapy has become much more efficacious with the introduction of anti-TNFs and the use of combination therapy, many patients still experience insuf-

ficient improvement on these agents. We are currently limited in option for patients who fail to respond to anti-TNF agents. In the near future, our patients may have access to IL-12/23 antibodies in CD, vedolizumab in CD and UC, possibly tofacitinib in both UC and CD. The promise of these agents is a bright light on the horizon for treatment of IBD.

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Prevention of post-operative recurrence of Crohn's disease

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Core tip: This review summarizes and updates the current state of the field of post-operative prevention of Crohn's disease (CD) after surgery. This review starts by discussing the natural history of postoperative recurrence followed by a summary of the most consistent and evidence based risk factors. We then discuss the evidence for medical prophylaxis of CD highlighting new data regarding biologics. Finally we discuss cost effectiveness and provide a potential novel treatment algorithm for a clinician to use practically when caring for a patient with CD after surgery.

Abstract

Endoscopic and clinical recurrence of Crohn's disease (CD) is a common occurrence after surgical resection. Smokers, those with perforating disease, and those with myenteric plexitis are all at higher risk of recurrence. A number of medical therapies have been shown to reduce this risk in clinical trials. Metronidazole, thiopurines and anti-tumour necrosis factors (TNFs) are all effective in reducing the risk of endoscopic or clinical recurrence of CD. Since these are preventative agents, the benefits of prophylaxis need to be weighed-against the risk of adverse events from, and costs of, therapy. Patients who are high risk for post-operative recurrence should be considered for early medical prophylaxis with an anti-TNF. Patients who have few to no risk factors are likely best served by a three-month course of antibiotics followed by tailored therapy based on endoscopy at one year. Clinical recurrence rates are variable, and methods to stratify patients into high and low risk populations combined with prophylaxis tailored to endoscopic recurrence would be an effective strategy in treating these patients.

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INTRODUCTION

Patients with Crohn's disease (CD) have a high likelihood of requiring surgery over their lifetime. Reasons for surgical intervention include failure of medical therapy, or a complication of stricturing or penetrating disease^[1]. It is estimated that half of patients will require surgery within 10 years of diagnosis^[2,3] and around 80% of patients will require surgery at some time in their life^[4-6]. Unfortunately, surgery for CD is not curative and recurrence is typically the rule. Symptomatic recurrence varies from 40%-80%^[5,7]. Endoscopic recurrence is much higher with up to 90% having lesions at 5 years^[4,7,8].

While the overall recurrence rates are high, the severity of recurrence varies amongst individuals. Some patients

may only develop mild endoscopic recurrence, and have no symptoms, whereas others may present soon after surgery with recurrent inflammation and clinical symptoms. Interventional studies have measured a drug's ability to prevent endoscopically-visible ulcers, or radiologically-apparent luminal narrowing, or symptoms. Assessing for severe endoscopic recurrence is useful, although still a surrogate predictor of future clinical recurrence. The wide variation of patients and outcomes used in clinical trials makes this topic difficult for the treating physician to apply to a given patient. The aim of this review is to summarize the literature regarding the medications available for prevention of post-operative recurrence as well as to discuss the different strategies for treating postoperative recurrence.

NATURAL HISTORY OF POST-OPERATIVE CROHN'S

The natural history of CD generally follows phenotypic patterns, whether in the pre-operative or post-operative patient. As stated above, recurrence in general is common both endoscopically and symptomatically after surgery. Crohn's recurrence after ileocecal resection or colectomy with ileorectal anastomosis tends to favor the anastomosis site, specifically the small bowel proximal to the anastomosis^[9]. Initially aphthous ulcers develop which then progress to larger ulcers and eventually complications of recurrent CD^[7,8]. It was noted in the late 1980s that patients who underwent surgery for a perforating phenotype of disease had a different post-operative course than those who underwent surgery for non-perforating indications^[10]. This group examined 770 patients who underwent surgery for CD at Mt. Sinai Hospital in New York. They found that not only did patients have a different course depending on their pre-operative phenotype, but they found that patients who required second and third operations, tended to do so for the same initial indication. Thus, patients who required an initial operation for penetrating disease tended to require a second, and potentially third, operation for penetrating disease. Thus overall the site and prevailing phenotype are thought to be similar before and after resection^[11].

An interesting observation into the pathogenesis of post-operative CD is that the fecal stream is needed for recurrence. Rutgeerts *et al.*^[8] reported on five patients who underwent ileal resection with ileocolonic anastomosis and additionally had a temporary, diverting ileostomy approximately 25-30 cm from their anastomosis site. At six months time, the anastomoses of these patients were compared to 75 other patients who underwent ileocolonic anastomosis without diverting ileostomy. None of the patients with the diverting ileostomy developed endoscopic or histologic changes consistent with CD compared to 71% of patients in the control group who had endoscopic recurrence at six months. All five patients however, did develop endoscopic recurrence of disease

at the anastomosis six months after the diverting ileostomy was taken down^[12]. Further study demonstrated that contact with intestinal fluid for only eight days was sufficient to trigger early histologic changes in a previously normal neo-terminal ileum and anastomosis^[13]. Within three months after ileocectomy the neoterminal ileum has been shown to have an increase in colonization with colonic flora, specifically *Escherichia coli* (*E. coli*), *Enterococci*, *Bacteroides* and *Fusobacteria* spp^[14]. Animal studies have also concluded that the presence of commensal bacteria is required for post-operative recurrence to develop^[15]. It remains unclear if these changes have a causative role in post-operative Crohn's recurrence, but they have been implicated in IBD *per se*^[16]. Thus, post-operative CD is associated with alterations in the local microbiome, development of local ulcers (endoscopic recurrence) and subsequent resumption of clinical recurrence.

RISK FACTORS FOR RECURRENCE: RISK STRATIFICATION

Multiple risk factors have been identified for postoperative recurrence. Risk stratifying patients based on these factors can thus help decide which patients to treat aggressively. Unfortunately, few risk factors have been consistent in the literature for predicting endoscopic, clinical or surgical recurrence. Typically risk factors are divided into patient-related factors, disease-related factors and surgical-related factors. Of the patient related factors, only smoking has consistently been identified as a predictor of postoperative recurrence. The odds ratio for clinical recurrence for current smokers is between 2 and 3^[4,17,18]. It is the only modifiable risk factor and smoking cessation appears to decrease the risk of recurrence to that of a non-smoker^[19]. Other patient related factors such as age, sex, age at disease onset are inconsistent risk factors in the literature^[20,21].

Disease related factors include duration of disease prior to first resection, history of previous resection and penetrating disease^[4,22]. Duration of disease prior to first surgery is not a consistent risk factor in the literature^[23,24]. Most studies demonstrate that previous surgery for CD is a risk factor for further surgery^[11,22,25]. A recent meta-analysis demonstrated a hazard ratio of 1.5 for recurrence of disease for penetrating phenotype compared to non-penetrating^[26]. Unfortunately, there was significant heterogeneity in the studies that went into the meta-analysis and thus it is not entirely clear what the relationship between penetrating disease and post-operative recurrence truly is^[21]. Further complicating the issue is that penetrating and stricturing disease are not always accurately diagnosed on clinical grounds. Up to a third of patients who clinically have non-penetrating disease may have evidence of penetration at the time of an operation^[27].

Surgical factors including length of resection, type of anastomosis and histological disease left in the bowel or granulomas in the resected specimen have not been

Table 1 Risk factors for postoperative recurrence

Risk factor	Strength of association (OR)	Grade of evidence ¹
Active smoking	2 (1.3-3.4) ^[18]	B
Penetrating disease	1.5 (1.2-1.9) ^[26]	B
Prior resection	1.8 (1.1-2.9) ^[22]	B
Myenteric plexitis	1.9 (1.0-3.5) ^[33]	C

¹Based on American Gastroenterological Association guidelines. A: Multiple, well-designed randomized controlled trials; B: At least one large well-designed clinical trial with or without randomization; C: Based on clinical experience, expert committees or descriptive studies.

consistently demonstrated to be risk factors for disease recurrence^[28-32]. However, myenteric plexitis in the resection specimen has been found to be predictive of postoperative recurrence^[33-35]. One small study found plexitis to be associated with prior intestinal surgery and shorter duration of disease thus potentially accounting for some of the variability of those risk factors as predictors of disease^[36]. Select risk factors that have been consistent in the literature are shown in Table 1.

MEDICAL THERAPIES TO PREVENT RECURRENCE

Multiple medications used for the treatment of Crohn's disease have been studied for the prevention of post-operative recurrence. Table 2 summarizes the endoscopic and clinical recurrences rates *vs* placebo from various trials.

5-ASA

The use of mesalamine in the postoperative setting is appealing given its favorable safety profile, ease of administration, and relatively lower costs to anti-TNFs. However controversy exists over its efficacy. McLeod *et al*^[31] demonstrated a 10% decrease in symptomatic recurrence ($P = 0.03$) with mesalamine at 3.0 g per day. Others have noted a decrease in endoscopic recurrence with varying mesalamine formulations^[37,38]. Despite this, some small prospective and retrospective studies failed demonstrate a difference compared to placebo^[39,40]. Interestingly, Hanauer *et al*^[41] found that postoperative mesalamine demonstrated a non-significant trend towards a clinical improvement compared to placebo, but did not have any effect on endoscopic or radiologic recurrence. A meta-analysis of available prospective studies did demonstrate that mesalamine decreased clinical as well as severe endoscopic recurrence at 12 mo with a NNT of 8 (RR *vs* placebo: 0.76, 95%CI: 0.62-0.94)^[42].

Thiopurines

Azathioprine and 6-mercaptopurine are efficacious in maintenance of CD and have been extensively studied in the post-operative setting. Only two studies have compared to a thiopurine to placebo and one was in combination with three months of metronidazole^[41,43]. Both trials showed a decrease of endoscopic recurrence in the

Table 2 Recurrence rates with each agent discussed (range) from randomized controlled trials, *vs* placebo rates

Agent	Endoscopic recurrence		Clinical recurrence	
	Drug	Placebo	Drug	Placebo
Mesalamine	5%-51%	50%	11%-16%	19%-23%
Thiopurines	2%-22%	50%	0%-36%	10%-50%
Anti-TNF	0%-21% ¹	81%-85% ¹	0%-20%	25%-46%
Antibiotics	13%	43%	7%-24%	25%-38%
Probiotics ²	9%-21%	15%-16%	9%-17%	6%-14%
Budesonide	52%	57%	57% ³	70% ³

Ranges of reported endoscopic and clinical recurrences for various therapies and the range of placebo reports from those trials. Endoscopic recurrence rates are for severe (\geq i2) recurrence unless otherwise stated. Follow-up time is 12 mo unless otherwise stated. ¹Any endoscopic recurrence; ²Three to twelve months follow-up; ³Combined clinical and endoscopic recurrence. TNF: Tumour necrosis factor.

thiopurine arm at 12 mo. Pooling the data demonstrated a favorable risk ratio for decreasing both clinical (RR *vs* placebo: 0.59, 95%CI: 0.38-0.92) and severe endoscopic recurrence (RR *vs* placebo: 0.64, 95%CI: 0.44-0.92) with thiopurines compared to placebo^[42].

Other trials have compared thiopurine to mesalamine formulation. However, superiority of a thiopurine over mesalamine was unable to be shown in multiple trials^[41,44-47]. It is possible that side effects often prohibit adherence of thiopurines^[46]. The meta-analysis by Doherty *et al*^[42] reported a higher risk of treatment discontinuation due to side-effects in patients taking thiopurines. Long term data from a retrospective analysis demonstrated that thiopurine treatment for over 36 mo decreased surgical recurrence compared to treatment less than 36 mo or no treatment at all^[48]. Thus long-term maintenance may be beneficial especially in those who can tolerate the treatment, but the side effect profile may be prohibitive especially as mesalamine agents may provide a similar decreased risk of recurrence.

Anti-TNF antibodies

Most recently anti-TNF therapeutic antibodies have been studied in the prevention of post-operative prophylaxis. Infliximab has been studied most, although there is some emerging data for adalimumab. Early non-controlled reports noted a decreased in both endoscopic and clinical recurrence of CD postoperatively^[49]. Subsequent prospective controlled studies also demonstrated a benefit compared to placebo^[50,51], mesalamine^[28,52] and thiopurines^[28]. However, there have only been two randomized trials comparing infliximab and both were compared with placebo. Regueiro *et al*^[53] demonstrated that endoscopic lesions were significantly lower at one year in the infliximab group compared to controls (9.1% *vs* 84.6% respectively, $P = 0.0006$), but were unable to detect a significant difference in the clinical recurrence rate due to small numbers. A follow up abstract from the same group suggested that patients can have a benefit for up to two years, although they will relapse if infliximab is stopped^[54]. Yoshida *et al*^[55] did demonstrate a decrease in

the clinical remission rate at 12 mo with infliximab compared to placebo (100% *vs* 68% respectively, $P < 0.03$). Additionally adalimumab has demonstrated efficacy if prevention of postoperative recurrence although data remains small with a lack of controls^[56-58]. Preliminary data from the POCER study was presented in abstract form demonstrated that at 6 mo 94% of high-risk patients treated with postoperative adalimumab were in endoscopic remission (i0-i1) *vs* 62% of high risk patients on a thiopurine ($P = 0.02$)^[59]. However, patients on a thiopurine who had recurrence had an escalation of therapy to adalimumab. Thus at 18 mo, there was no difference between endoscopic recurrence in patients who received adalimumab immediately post operatively and those who had tailored therapy based on 6 mo endoscopy^[60]. Thus early data from ongoing trials suggests that prophylaxis with anti-TNF antibodies may be highly effective compared to other treatments, although careful patient selection is likely required to identify whom to administer prophylaxis to.

Probiotics

Manipulation of the bacterial flora is an attractive mode of preventing postoperative recurrence, as specific bacteria including bacteroides, fusobacteria and *E. coli* have been found in increased amount in the neo-terminal ileum^[14]. Both individual *Lactobacillus* strains as well as probiotic cocktails have been studied. *Lactobacillus johnsonii* (LA1) and *Lactobacillus rhamnosus* GG (LGG), failed to show any benefit in prevention of recurrence^[61-63]. The probiotic cocktail Synbiotic 2000 similarly failed to show a benefit compared to placebo in a randomized controlled trial^[64]. Similarly the yeast *Saccharomyces boulardii* did not demonstrate prevention of relapse in a randomized controlled trial of patients in medical remission^[65]. However, a small study with VSL#3 did note that patients receiving the probiotic had less severe endoscopic recurrence (lower Rutgeerts score) and reduced levels of pro-inflammatory cytokines^[66]. Ultimately, pooled data failed to show any difference with regards to clinical (RR *vs* placebo: 1.41, 95%CI: 0.59-3.36) or endoscopic recurrence (RR *vs* placebo: 0.96, 95%CI: 0.58-1.58)^[42].

Antibiotics

Nitroimidazole antibiotics including metronidazole and ornidazole have been shown to decrease the risk of clinical and severe endoscopic recurrence^[42,67,68]. Further illustrating the usefulness of antibiotics was a trial where all patients received metronidazole for one month and either azathioprine or placebo for one year. The overall rate of recurrence was low which was attributed to the widespread metronidazole use, although in combination with azathioprine endoscopic recurrence was less frequent and less severe^[43]. However, nitroimidazole antibiotics are difficult to tolerate and cessation rates are high, up to one third, limiting widespread clinical utility^[67,68].

COSTS AND DURATION OF CHEMOPROPHYLAXIS

The goal of therapy for post-operative prophylaxis is to decrease clinical recurrence. Not all patients will develop clinical recurrence and so treating everyone one may not be cost effective or feasible. Two studies examined the cost and feasibility of treating patients with various medical therapies in the postoperative setting. Doherty *et al*^[69] noted that incremental cost-effectiveness ratio (ICER), or costs per QALY gained, was \$1.8M for infliximab, when compared with no prophylaxis. In their cost-effectiveness model, azathioprine was the most cost-effective medication strategy over 1 year, when compared to "no prophylaxis". Ananthakrishnan *et al*^[70] similarly noted that initial therapy with infliximab is not cost effective although suggested that antibiotics (metronidazole or ornidazole) were cost effective when tolerated. Both of these studies made assumptions of a recurrence rate of around 20%-30% with no treatment. As the rate of recurrence increases, the cost-effectiveness of an anti-TNF increases as well.

USING ENDOSCOPIC SCREENING TO TAILOR INTERVENTIONS

Endoscopic recurrence precedes clinical recurrence, but not all endoscopic recurrence leads to clinical recurrence. Endoscopic changes are often noted as early as 2 mo following surgery^[71]. Endoscopic recurrence rates vary widely at 1 year without any treatment ranging from 28%-93%^[72]. Overall, it is estimated that 20% of patients with endoscopic recurrence will progress to clinic recurrence, although the severity of the endoscopic recurrence can predict the likelihood of clinical recurrence^[8,19]. Over a four year follow-up period, 100% of patients with severe endoscopic recurrence (Rutgeerts score of i2-i4) developed symptomatic recurrence compared to only 9% of patients with a low score (i0-i1)^[73]. Thus evaluation of a patient within the first year following resection of CD can effectively stratify patients at a high risk of clinical recurrence. Patients who develop a high Rutgeerts score ($\geq i2$) who are then treated with infliximab have decreased clinical activity compared to treatment with thiopurines or mesalamine^[28]. One study demonstrated that an endoscopically-tailored therapy (with any medical therapy) has a similar time to symptomatic recurrence compared to immediate post operative prophylaxis^[74]. Recently, preliminary results of the POCER study were presented, supporting the use of endoscopically tailored therapy. In this trial, all patients received three months of antibiotics and high-risk patients (smoker, penetrating disease, \geq second operation) received a thiopurine (or every other week adalimumab if thiopurine intolerant). Patients were then randomized to active care with a colonoscopy at 6 mo and step up of therapy if evidence of recurrence (Rutgeerts score $\geq i2$) or standard of care. At

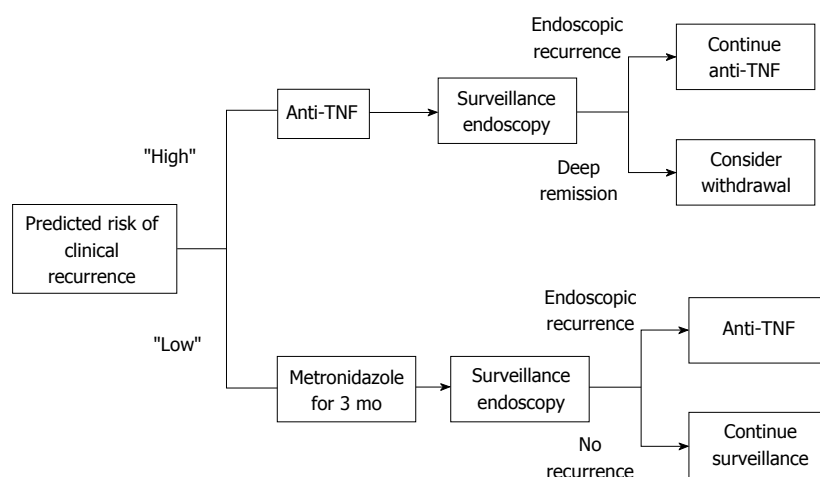


Figure 1 Suggested algorithm for deciding when to administer post-operative prophylaxis based on effectiveness of treatment. Other considerations such as cost and prior success or failure of treatment need to be individualized. Patients at high risk are those who have 2 or more of the following risk factors: smoking, penetrating disease, history of prior resection, and myenteric plexitis. Based on the available evidence, we suggest first surveillance endoscopy be done at 6 mo time for all patients. Treatment escalation at 6 mo should be considered for all patients with evidence of endoscopic recurrence (Rutgeerts \geq i2). For select patients who achieve and maintain deep remission on therapy (Rutgeerts of i0 with normal histology), consideration can be given to de-escalation of therapy to either thiopurine alone or close monitoring.

18 mo, significantly less endoscopic recurrence was seen in the active care group *vs* the standard of care group (49% *vs* 67% respectively, $P = 0.028$)^[75].

An interesting option to explore in the future is immediate prophylaxis followed by endoscopic assessment and cessation of treatment if no endoscopic recurrence or other surrogate marker for inflammations such as serum inflammatory markers or fecal calprotectin. One retrospective cohort demonstrated that patients who achieve clinical remission on infliximab and maintain maintenance therapy for one year have a 69% probability of being disease free at 1 year after infliximab discontinuation^[76]. The STORI trial is a prospective cohort following a group of patients who have received > 1 year of therapy with infliximab and an immunomodulator and achieved steroid free remission who then stop infliximab. Over 50% of patients will not relapse at one year and there may be a subgroup of patients in deep remission who do even better^[77]. Extrapolating this premise and applying it to patients who achieve surgical remission followed by a year of therapy is appealing and potentially cost effective. One small study has evaluated one year of infliximab for chronic refractory pouchitis following colectomy for ulcerative colitis. Notably the patients who responded completely to infliximab were able to discontinue after one year without recurrence at follow-up^[78].

IMPLICATIONS FOR PRACTICE

The questions of if and when to treat in the post-operative setting, as well as the optimal therapeutic regimen, remains incompletely answered. Each patient requires an individual risk assessment as well as careful consideration to prior therapies and what led them to surgery^[4]. Overall, tailored therapy is advisable as a general rule given the cost and side effect profile of the majority of

treatments. However, in the highest risk patients (*i.e.*, penetrating disease, history of prior resection, current smoking), upfront infliximab use provides the greatest benefit although it is not considered cost effective^[70]. As long-term data on infliximab becomes available, the balance of cost and prevention of disease may change. Additionally as data becomes available for other anti-TNFs, this model will need to be updated. Thus for the highest risk patients, the decision for upfront anti-TNF *vs* tailored therapy remains unclear and must likely be individualized. If the decision for tailored therapy is made, then endoscopic evaluation should take place at 6 mo time as suggested by the recent POCER data^[60,75]. In patients who are not high risk, then an empiric course of nitroimidazole antibiotic is likely to be cost effective, if the patient can tolerate the therapy, with or without maintenance thiopurine followed by endoscopic assessment at 6 mo time^[4,60,70,75]. A proposed algorithm for post-operative prevention is shown in Figure 1. While this approach is the most efficacious based on the available evidence, it is not the most cost effective and the decision for upfront anti-TNF therapy or tailored therapy in high risk patients will need to be individualized.

The optimal treatment regimen is not known and likely will depend heavily on the patient's prior treatments. The biologic naïve patient may achieve the best effect with an anti-TNF, although the cost effectiveness of long-term prevention may not be favorable. While the majority of data is with infliximab, it appears that adalimumab will be efficacious as well, necessitating further cost effective analysis. Postoperative prophylaxis in CD does decrease clinical recurrence. However the optimal timing and type of therapy remain unknown. Stratifying patients according to risk of symptomatic recurrence and tailoring therapy is the ideal and most cost effective way to treat patients, however these questions have not been fully answered.

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Role of capsule endoscopy in inflammatory bowel disease

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Abstract

Videocapsule endoscopy (VCE) has revolutionized our ability to visualize the small bowel mucosa. This modality is a valuable tool for the diagnosis of obscure small bowel Crohn's disease (CD), and can also be used for monitoring of disease activity in patients with established small-bowel CD, detection of complications such as obscure bleeding and neoplasms, evaluation of response to anti-inflammatory treatment and postoperative recurrence following small bowel resection. VCE could also be an important tool in the management of patients with unclassified inflammatory bowel disease, potentially resulting in reclassification of these patients as having CD. Reports on postoperative monitoring and evaluation of patients with ileal pouch-anal anastomosis who have developed pouchitis have recently been published. Monitoring of colonic inflammatory activity in patients with ulcerative colitis using the recently developed colonic capsule has also been reported. Capsule endoscopy is associated with an excellent safety profile. Although retention risk is increased in patients with small bowel CD, this risk can be significantly decreased by a routine utilization of a dissolvable patency capsule preceding the ingestion of the diagnostic capsule. This paper contains an

overview of the current and future clinical applications of capsule endoscopy in inflammatory bowel disease.

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Key words: Small bowel videocapsule endoscopy; Crohn's disease; Pouchitis; Indeterminate inflammatory bowel disease; Ileal pouch-anal anastomosis; Patency capsule

Core tip: Videocapsule endoscopy has revolutionized our ability to visualize the small bowel mucosa. This modality is a valuable tool for the diagnosis of obscure small bowel Crohn's disease (CD), and can also be used for monitoring of disease activity, detection of complications, evaluation of therapeutic response and postoperative recurrence in established CD, evaluation of the small bowel in patients with unclassified inflammatory bowel disease and pouchitis. Monitoring of colonic inflammation in patients with ulcerative colitis has also been reported. This manuscript contains an overview of the current and future clinical applications of capsule endoscopy in inflammatory bowel disease.

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INTRODUCTION

In the past, the small bowel has largely been inaccessible to direct endoscopic examination, with only the duodenum, proximal jejunum and terminal ileum being subject to direct visualization by a conventional endoscope. This paradigm changed dramatically with the invention and introduction of small bowel videocapsule endoscopy

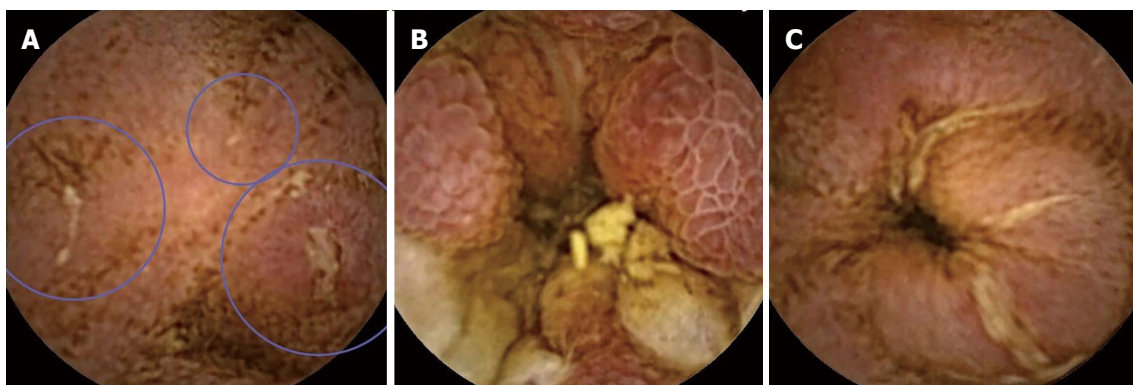


Figure 1 Videocapsule endoscopy findings. A: Small ulcers (encircled); B: Edematous mucosa; C: Ulcerated stenosis (SBII capsule, RAPID and Imaging software, Given Imaging, Yokneam, Israel).

(VCE) in 2000^[1]. The first wireless capsule, manufactured by Given Imaging (Yokneam, Israel) was approved for clinical use in United States and Europe in 2001^[2]. Several other manufacturers subsequently released their own versions of VCE. This technology has been extensively used for the diagnosis and monitoring of patients with inflammatory bowel disease (IBD), mostly Crohn's disease (CD). About 30% of the patients with CD have exclusive small bowel involvement^[3], and their diagnosis will frequently be missed if based solely on ileocolonoscopy findings. VCE is now also considered an important technique for monitoring small bowel CD, and has also been employed in management of patients with unclassified IBD and ulcerative colitis.

The aim of the current review is to outline the diagnostic role of VCE in the diagnosis and monitoring of inflammatory bowel disease, in particular small-bowel CD.

DIAGNOSIS OF CD

Characteristic endoscopic findings

Several VCE findings are frequently associated with CD: ulcerations, erythema, mucosal edema, loss of villi, strictures and mucosal fissures (Figure 1)^[4]. Unfortunately, none of these findings is specific for CD. In fact, minor small bowel lesions may be present in up to 10% of normal subjects^[5]. As VCE lacks tissue-sampling capabilities, it cannot confirm the etiology of the observed lesions. The most common mimicker of CD in the small bowel is non-steroidal anti-inflammatory medication (NSAID)-induced enteropathy that may manifest with lesions indistinguishable from those of CD. Such lesions, appearing as early as 2 wk from the onset of NSAID therapy, can be demonstrated in 70% of chronic NSAID users^[6,7]. Thus, VCE should be reserved for patients with high clinical index of suspicion for CD. Patients who are candidates for VCE should be instructed to avoid NSAIDs for at least 1 mo before the examination. Similar bowel mucosal lesions may result from multitude of other pathologies, such as lymphoma, radiation enteritis, HIV with opportunistic infection, intestinal tuberculosis and Behcet's disease^[5].

Diagnostic scores

The criteria for diagnosis of CD using VCE have not been well established. The most commonly used validated diagnostic index is the Lewis score^[8]. This score divides the small bowel into 3 tertiles (dividing the small bowel transit time in 3) and uses an algorithm that assigns points to various findings (mucosal edema, ulcers, strictures) characteristic for CD in each of the tertiles, taking in account the severity and the reproducibility of each finding. The final score represents the number of points accumulated by the most significantly involved tertile. The Lewis score is incorporated in the software used for decoding, reading and interpretation of VCE images obtained by PillCAM (RAPID). A score < 135 is designated as normal or clinically insignificant mucosal inflammatory changes, a score between 135 and 790 indicates mild, and a score ≥ 790 moderate to severe inflammation, respectively. An additional score known as capsule endoscopy CD activity index (CECDAI or Niv score), was recently proposed (Table 1)^[9]. This score incorporates three main characteristics of CD: inflammation, extent of disease, and strictures, in both the proximal and distal segments of the small bowel. It should be noted that while these scores attempt to quantify the severity and extent of small bowel (SB) CD, the lesions are not pathognomonic and may represent other causes of bowel inflammation.

VCE was also utilized for diagnosis of SB CD in patients primarily presenting with extraintestinal manifestations of IBD. Arthropathy is the most common extraintestinal manifestation in IBD, occurring in 6%-46% of the patients^[10], and frequently manifesting even before the onset of bowel disease. Capsule endoscopy may be a valuable tool in evaluation of these patients, especially if conventional ileocolonoscopy is unappealing to the patient^[11]. Spondyloarthropathy can be diagnosed in up to 30% of IBD patients^[12,13]. Capsule endoscopy can demonstrate small bowel lesions consistent with CD in 33% of these patients (twice as many as conventional ileocolonoscopy^[14]).

VCE vs other modalities for the diagnosis of CD

The yield of VCE for the diagnosis of CD was com-

Table 1 A comparison of 2 capsule endoscopy scoring indices for quantification of mucosal inflammation

Parameter	Lewis score ^[8]			CECDAI ^[80]	
	Number/quality	Longitudinal extent	Descriptors	Parameter	Descriptors
Villous appearance	Normal/edematous	Short segment/long segment/whole tertile	Single/patchy/diffuse	Inflammation score	None to large ulcer (> 2 cm)
Ulceration	Non/single/few/multiple	Short segment/long segment/whole tertile	< 25%, 25%-50%, > 50%	Extent of disease	No disease to diffuse (3 segments)
Stricture	Non/single/few/multiple	Ulcerated/non-ulcerated	Traversed/non-traversed	Stricture score	None to complete obstruction
Small bowel segmentation	Tertiles (strictures for the entire length of the examination)			Proximal to distal small bowel	
Score	< 135: Normal or clinically insignificant inflammation 135-790: Mild inflammation 790: Moderate to severe.			0 (normal examination)-26 (severe inflammation)	

CECDAI: Capsule endoscopy Crohn's disease activity score.

Table 2 Key studies evaluating the diagnostic yield of capsule endoscopy for Crohn's disease

Modality	Ref.	Number of patients	Diagnostic yield of VCE	Diagnostic yield of the compared modality	IY	P value
CTE	Eliakim <i>et al</i> ^[81]	35	77%	20%	47%	< 0.05
	Hara <i>et al</i> ^[82]	17	71%	53%	18%	NA
	Voderholzer <i>et al</i> ^[83]	41	61%	49% (CT enteroclysis)	12%	< 0.04
	Solem <i>et al</i> ^[84]	40	83%	83%	0	NS
MRE	Albert <i>et al</i> ^[85]	27	93%	78%	15%	NS
	Crook <i>et al</i> ^[22]	19	93%	71%	18%	NS
	Jensen <i>et al</i> ^[20]	93	100%	86%	14%	NS
	Hara <i>et al</i> ^[82]	17	71%	65%	6%	NS
Ileocolonoscopy	Solem <i>et al</i> ^[84]	40	83%	74%	9%	NS
	Leighton <i>et al</i> ^[86]	80	55%	25%	30%	NA

VCE: Videocapsule endoscopy; CTE: Computed tomography enterography; MRE: Magnetic resonance enterography; IY: Incremental yield; NA: Not available; NS: Non significant.

pared to that of cross-sectional imaging modalities such as small bowel follow-through (SBFT), computer tomography enterography (CTE) and magnetic resonance enterography (MRE) in multiple studies (Table 2). Patients with suspected small bowel stenosis were excluded from VCE evaluation in these studies. The superiority of VCE over small bowel follow-through and enteroclysis has been repeatedly demonstrated in multiple studies^[15-18].

A recent meta-analysis demonstrated an incremental diagnostic yield (IY) of VCE in comparison to CTE in both suspected and established CD patients (IY, 47%; 95%CI: 31%-63%, $P < 0.00001$; and 32%; 95%CI: 16%-47%, $P < 0.0001$), respectively^[19]. A prospective trial evaluated the diagnostic accuracy of VCE, MRE and CTE in 93 patients with suspected CD as compared to ileocolonoscopy. The sensitivity and specificity for diagnosis of CD of the terminal ileum was 100% and 91% by CE, 81% and 86% by MRE, and 76% and 85% by CTE, respectively. There was statistical difference in sensitivity compared with CTE, but only a trend in comparison with MRE. Specificity was not significantly different between the modalities. Proximal small bowel CD was detected in 18 patients by using CE, compared with 2 and 6 patients using MRE or CTE, respectively ($P < 0.05$)^[20]. In earlier studies, VCE and MRE were reported to have comparable

accuracy. Overall, VCE is more accurate in diagnosing subtle small bowel lesions and MRE in diagnosing intramural inflammation, stricturing complications and extra-intestinal manifestations^[19,21,22]. The superior sensitivity of VCE clinical for proximal small bowel disease is a potentially important diagnostic advantage, as proximal small bowel disease has recently been demonstrated to be a significant negative prognostic factor^[23].

Importantly, data acquired by different endoscopic and imaging modalities can be combined to improve the diagnostic accuracy, utilizing the specific advantages and strengths of each modality.

VCE in established CD

VCE is a potentially important but currently underutilized tool for monitoring of SB CD. In the latter years, the leading treatment paradigm in IBD has shifted from merely controlling symptoms to reversing the underlying inflammation, as expressed by objective surrogate markers such as laboratory inflammatory markers and endoscopic evidence of mucosal healing^[24]. Capsule endoscopy provides meaningful information on the inflammatory burden in the small bowel mucosa, similarly to the role of conventional ileocolonoscopy for the colon and the terminal ileum. Bowel stenosis should be ruled

Table 3 Key studies describing the role of videocapsule endoscopy in established Crohn's disease

Indication	Ref.	n	Inclusion criteria	Diagnostic criteria	Results
Mucosal healing	Efthymiou <i>et al</i> ^[28]	40	Patients with active CD (CDAI > 150) who responded to anti-inflammatory treatment, VCE was performed before and after treatment	Number of aphthous ulcers/large ulcers/length of involved segment	Only number of large ulcers correlated with response (8.3 ± 1.4 and $5/0.8$, 95%CI: 0.8-5.9, $P < 0.01$)
Postoperative recurrence	Bourreille <i>et al</i> ^[31]	31	CD with ileocolonic anastomosis	Rutgeerts score ≥ 1	VCE-21/31 (68%), IC-19/31 (61%)
	Pons Beltrán <i>et al</i> ^[32]	24	CD with ileocolonic anastomosis	Rutgeerts score ≥ 2	VCE-14/22 (55%), IC-6/24 (25%)
Unexplained symptoms	Dubcenco <i>et al</i> ^[34]	28	Active CD patients	≥ 3 ulcers	VCE-23 (82%), IC-14 50%, barium radiography-9 (32%)
	Dussault <i>et al</i> ^[35]	25	Active CD patients with unexplained symptoms	Severity graded by number and appearance of ulcers and presence of stenosis	Active SB inflammation: 11/25 (44%)

In 6 patients treated with immunomodulators, biologics or corticosteroids, a significant improvement was demonstrated in all 3 parameters. CD: Crohn's disease; VCE: Videocapsule endoscopy; IC: Ileocolonoscopy; SB: Small bowel.

out before VCE is performed in established CD due to the increased risk of capsule retention (about 5%). Routine use of patency capsule diminishes the risk of retention to almost negligible (see below).

VCE could be particularly useful in the following clinical scenarios in known CD (Table 3): (1) Monitoring of mucosal healing; (2) Detecting postsurgical recurrence; and (3) Discrepancy between clinical and laboratory data and endoscopic findings.

Mucosal healing: Mucosal healing, defined as absence of visible endoscopic inflammation, has emerged as a very important marker of long-term clinical efficacy associated with decreased risk of long-term complications in both ulcerative colitis (UC) and CD^[24-27]. Conventional ileocolonoscopy is the current gold-standard modality for assessment of mucosal healing. A small prospective study had evaluated monitoring of mucosal healing with VCE performed before and after treatment for acute CD flare-up^[28]. Forty patients with CD flares were included in the study and all have responded to treatment within 4-8 wk of treatment. Three parameters (number of large ulcers, number of aphthous ulcers and percentage of time with lesions visible) were examined. Of these only the first one improved significantly. In a subgroup of patients treated with corticosteroids combined with immunomodulators or biologics, a significant improvement in all three parameters was demonstrated. The most important limitations of this study were a significant heterogeneity in the instituted treatment, with majority of patients treated with mesalamine or corticosteroids, along with absence of a validated scoring system for mucosal inflammation. The data from our center demonstrated a significant reduction in the Lewis score in 4 patients with spondyloarthropathy and newly diagnosed SB CD after 6 mo of treatment with Adalimumab^[14]. Importantly, no diagnostic score, including the commonly used Lewis score, has been validated for evaluation of mucosal healing in SB CD.

Postoperative CD recurrence: Recurrence of SB CD in the neo-terminal ileum following surgical resection can

be demonstrated in 73%-93% of the patients within 1 year of ileocolonic resection^[29,30]. SB lesions associated with postoperative recurrence are frequently quantified using the Rutgeerts score^[29]. The accuracy of VCE in detection of postoperative recurrence was evaluated in 31 patients^[31]. Recurrence occurred in 21 patients (68%) and was detected by ileocolonoscopy in 19 patients. Sensitivity of VCE using the Rutgeerts score was 62%-76% and specificity was 90%-100%. The severity of lesions as assessed by both methods correlated significantly ($P < 0.05$). In an additional study, 24 patients with CD, neo-terminal ileum recurrence defined as Rutgeerts score > 2 was demonstrated by ileocolonoscopy in 25% and capsule endoscopy in 62% (VCE was performed in 22/24 patients due to failure to excrete the patency capsule in 2 patients). Capsule endoscopy detected proximal SB lesions inaccessible by ileocolonoscopy in 13 patients^[32]. VCE is an attractive monitoring modality for postoperative patients, providing a non-invasive and accurate visualization of the entire small bowel including the neo-terminal ileum.

Unexplained symptoms: Many symptoms of CD, such as diarrhea, abdominal pain, bloating, can be attributed to a multitude of etiologies other than active inflammation [underlying irritable bowel syndrome (IBS), bacterial overgrowth, bile salt diarrhea *etc.*]. Clear identification of inflammatory etiology is of crucial importance and may lead to significant changes in the treatment, such as initiation or escalation of anti-inflammatory treatment. Negative VCE results are also of clinical importance, as this would lead to diagnosis and initiation of treatment for a concomitant condition such as IBS, and prevent further unnecessary and expensive escalation of an anti-inflammatory regimen. Clinical indices and laboratory inflammatory markers may indicate ongoing inflammation, but lack sensitivity. In a study including 140 patients with CD, the Spearman's rank correlation of simple endoscopic index with fecal calprotectin, CRP, blood leukocyte count and CDAI was 0.75, 0.53, 0.42 and 0.38, respectively^[33]. Although ileocolonoscopy is a gold standard test for identification of active inflammation, it would potentially miss lesions located proximally to the ileocecal valve.

Table 4 Key studies describing the role of videocapsule endoscopy in unclassified Inflammatory bowel disease pouchitis and ulcerative colitis

	Ref.	Indication	n	Definition of CD	Results n (%)
IBD-U	Mehdizadeh <i>et al</i> ^[41]	IBDU	6	> 3 ulcerations-diagnostic of CD, 1-2 ulcerations-suggestive of CD	1 (17) SB findings
	Maounoury <i>et al</i> ^[42]	IBDU	30	> 3 ulcerations-diagnostic of CD, 1-2 ulcerations-suggestive of CD	5 (17), CD
s/p IPAA	Cohen <i>et al</i> ^[43]	IBDU	2	NA	1 (50), CD
	Mow <i>et al</i> ^[40]	Isolated colitis	6	> 3 ulcerations-diagnostic of CD, 1-2 ulcerations-suggestive of CD	3 (50)-definite CD, 2 (20), possible CD
	Mehdizadeh <i>et al</i> ^[41]	Persistent symptoms after IPAA	21	> 3 ulcerations-diagnostic of CD, 1-2 ulcerations-suggestive of CD	7 (33%) SB findings
	Calabrese <i>et al</i> ^[47]	Chronic pouchitis after IPAA	15	NA	Gastric or SB lesions, 15 (100)
	Mow <i>et al</i> ^[40]	Isolated colitis	12	> 3 ulcerations-diagnostic of CD, 1-2 ulcerations-suggestive of CD	3 (25)-definite CD, 3 (25), possible CD
Ulcerative colitis	Mehdizadeh <i>et al</i> ^[41]	Treatment-resistant UC	22	> 3 ulcerations-diagnostic of CD, 1-2 ulcerations-suggestive of CD	2 (9), SB findings
	Cohen <i>et al</i> ^[43]	UC	5	NA	4 (80), CD
	Higurashi <i>et al</i> ^[44]	UC	23	Lewis score	13 (56.5), small bowel lesions 9 (39), Lewis score > 135

IBDU: Unclassified inflammatory bowel disease; IPAA: Ileoanal pouch anastomosis; CD: Crohn's disease; UC: Ulcerative colitis; SB: Small bowel.

Dubcenco *et al*^[34] have prospectively evaluated 28 symptomatic Crohn's patients with ileocolonoscopy, barium radiography and capsule endoscopy. Active disease was identified by VCE, ileocolonoscopy and barium radiography in 82%, 49% and 32% of patients, respectively. In a study by Dussault *et al*^[35], in 25 out of the included symptomatic CD patients, VCE was indicated for a discrepancy between clinical symptoms and diagnostic findings. Abnormal SB findings were diagnosed in 44% of the patients, and in 45% of these patients the treatment was escalated following the performance of VCE.

VCE can also be used for monitoring of ileal recurrence in CD patients following bowel resection and ileocolonic anastomosis. In one study, VCE detected CD recurrence in 15 (62%) patients, whereas ileocolonoscopy detected inflammatory lesions in the neo-terminal ileum in only 6 (25%) patients^[32]. VCE was also evaluated for a potential role in the assessment of mucosal healing after drug therapy in CD^[28].

Therapeutic yield of VCE in established CD

VCE frequently produces clinically significant data that can lead to a change in a therapeutic management. In a retrospective series of 71 CD patients, medical treatment was changed in 38 (53%) of the patients within 3 mo of VCE performance^[35]. In an additional series that included 86 patients with established CD, 61.6% had a change in medication in the 3 mo after the CE, with 39.5% initiating a new anti-inflammatory medication^[36].

VCE in unclassified IBD and UC

Colonic inflammatory bowel disease cannot be classified as CD or UC using current colonoscopic and pathologic criteria in 10%-15% of the patients^[37]. At least 30% of these patients with unclassified IBD (IBDU) will be reclassified as CD during the course of their illness^[38],

usually after identification of small bowel lesions. Correct classification of the patients is especially important when deciding on surgical intervention, as rates of chronic pouchitis, fistula formation and pouch failure after ileal pouch-anal anastomosis (IPAA) are significantly higher in patients with CD^[39].

Several small studies have evaluated the utility of VCE for reclassification of IBDU patients. Mow *et al*^[40] have described 22 patients with either isolated colitis or chronic symptoms following IPAA ($n = 18$) who were evaluated with VCE. All patients had prior unremarkable small bowel radiography. Multiple ulcerations (3 and above) considered diagnostic for CD were identified in 68 of 18 patients. Mehdizadeh *et al*^[41] described 120 patients with a history of UC or IBDU who underwent VCE. Findings consistent with SB CD were demonstrated in 15.8% of the patients. Eighteen/19 patients with CD diagnosed by VCE have previously underwent SBFT, with positive findings in only 1 patient. Another series included 30 patients with IBDU, in whom CD (defined as 3 or more SB ulcers) was identified in 5. Interestingly, in 6/25 VCE-negative patients CD was diagnosed on a subsequent ileocolonoscopy with biopsies^[42]. In a series of pediatric patients, 5/7 patients initially diagnosed as UC or IBDU were reclassified as having CD as a result of VCE findings^[43] (Table 4).

Higurashi *et al*^[44] evaluated small bowel inflammation in patients with established UC. Of the 23 UC patients, 13 (57%) showed small-bowel lesions, and 8 (35%) had erosions, as opposed to 2/23 (7%) and 1/23 (4%) in the control group. In 9/23 patients with UC, the Lewis score of inflammation was consistent with mild to moderate small bowel inflammation (between 135 and 790). The clinical and pathological significance of these lesions is unclear (repeated biopsies were performed in only 2 patients, but these results are of great interest and em-

phasize the possible risk of misdiagnosis in many IBD patients.

VCE in evaluation of pouchitis in patients after IPAA

IPAA provides a continence-preserving surgical option in patients with UC unresponsive or unwilling to continue anti-inflammatory therapy, or those who have developed complications (such as colonic stenosis, colonic dysplasia *etc.*) that require total colectomy. The procedure is technically demanding and is associated with a significant incidence of postoperative complications, the most common being chronic and acute pouchitis and “*de novo*” CD^[45]. Symptoms and endoscopic lesions consistent with chronic pouchitis are reported in 10%-59% in patients with UC, and even more frequently in patients with CD^[46]. It is commonly argued that at least a subgroup of these patients actually represent a previously undiagnosed CD. In a series of 15 UC patients with chronic pouchitis, diffuse lesions involving the stomach or different segments of the small bowel were demonstrated in all patients^[47]. Similar lesions were demonstrated in 27% of the control patients. Unfortunately, histological evaluation (showing non-specific inflammation) was available for only 2 patients with gastric involvement. The clinical significance of these lesions is unclear (Table 4).

The role of VCE in preoperative evaluation of UC/IBDU patients was examined in one retrospective series. The study evaluated the incidence of acute pouchitis, chronic pouchitis and *de novo* CD within 12 mo of the surgery in patients with and without pathological findings on a preoperative VCE. No significant difference was demonstrated for any of the outcomes^[48]. However, an important limitation of this study was the definition of “positive” VCE as any ulceration or lesion, possibly leading to a high false positive rate. In addition, a significant selection bias stemming from the retrospective design of the study (patients with a high preoperative probability of CD were not likely to undergo IPAA) interferes with the interpretation of the results. In our opinion, preoperative evaluation of IPAA candidates with VCE merits further evaluation in prospective studies.

Anemia is another frequent complication of IPAA surgery, occurring in about 17% of UC patients^[49]. Possible etiologies may include newly discovered CD, arteriovenous malformations, celiac disease and others. In a series of post-IPAA patients with chronic anemia, VCE detected the cause of anemia in 29.4%. Sixty percent of the patients were diagnosed as having a new-onset CD^[50].

COLONIC CAPSULE ENDOSCOPY IN UC

A colonic capsule [PillCam colonic capsule (PCCE) Given Imaging, Yokneam, Israel] has been available for colorectal cancer screening for several years. This device includes 2 cameras which records 2 different sets of images. The colonic capsule was compared with colonoscopy with promising results, with the second-generation capsule reaching sensitivity of 88% for detection of pol-

yps > 6 mm in comparison to colonoscopy^[51,52].

PCCE was evaluated for diagnosis and monitoring of UC. In the study by Ye *et al.*^[53], 25 patients were evaluated for presence and severity (Mayo Score) of UC by PCCE and conventional colonoscopy. A significant correlation in the severity ($k = 0.751$, $P < 0.001$) and extent ($k = 0.522$, $P < 0.001$) of UC between the PCCE and conventional colonoscopy was demonstrated. Similar findings were reported by Hosoe *et al.*^[54]. However, PCCE is not suitable for monitoring of dysplasia and cancer surveillance in UC patients due to its lack of tissue sampling ability.

Contraindications and risks

The main complication of CE is capsule retention, defined as a failure to excrete the capsule for 2 wk or more, requiring directed medical, endoscopic or surgical intervention^[55]. CE is contraindicated in patients with known bowel strictures or swallowing disorders, and history of bowel obstruction. Recent abdominal surgery is a relative contraindication^[56]. In patients with obstructive symptoms or one of the aforementioned risk factors, cross-sectional imaging should be performed before VCE; however, absence of strictures on cross-sectional imaging does not preclude capsule retention^[57]. The rate of capsule retention depends on the indication for performance of VCE^[58]: 0% in healthy controls^[59], 1.4% in obscure gastrointestinal bleeding^[60-62], 1.48% in suspected CD^[63-65], 5%-13% in known CD^[40,66] and 21% in suspected small bowel obstruction^[67]. Slow transit of the capsule, with delayed excretion of the capsule is very common, seen in up to 20% of the cases^[56]. A retained capsule is usually asymptomatic^[68], but may be associated with symptoms of partial or complete bowel obstruction. Only 6 cases of bowel perforation were reported^[56,69]. Usually, the retained capsule can be extracted with surgery or enteroscopy. If the cause is an inflammatory stricture, corticosteroids have been useful in some cases. No consensus on the timing of intervention exists, and it is unclear how long one should wait before intervention in asymptomatic patients.

Patency capsule

The patency capsule has the same shape and dimensions as the real videocapsule. It is constructed of cellophane with wax plugs at either end and it contains lactose mixed with 10% barium to make it radiopaque. The wax plugs have holes that allow succus entericus to dissolve the lactose, resulting in capsule disintegration^[70]. The dissolution of the patency capsule (Agile, Given Imaging) starts to occur after 30 h. The patency capsule can be detected by radiography or a portable radiofrequency scanner. When the patency capsule is successfully excreted or not detectable on radiography in the small bowel at 30 h post ingestion, it is usually safe to perform the diagnostic VCE. If the patency capsule location is uncertain, it is possible to localize it with the assistance of contrast or air enhanced fluorography or CT^[71]. The rate of excretion of the pa-

Table 5 Potential future technological developments in capsule endoscopy relevant to inflammatory bowel disease

Manually manipulated capsule ^[77]
Electrically propelled capsule ^[78]
Tissue sampling capabilities (brushing, cytology, fluid aspiration) ^[87]
Therapeutic capabilities (cautery) ^[87]
Drug delivery

tency capsule varies from 45%-88%^[58,72-75], depending upon patient selection. In a series of 77 CD patients who underwent a patency capsule examination before proceeding to diagnostic VCE, the patency capsule was not excreted within 30 h in 7.8% of the patients^[35]. The main complication of patency capsule is mild abdominal pain, occurring in about 20% of the patients. Clinically evident intestinal obstruction requiring surgical intervention was reported in very few cases^[58]. This phenomenon may be explained by the lodging of the capsule in sites of obstruction not easily assessable by intestinal fluids necessary for the dissolution of the lactose in the patency capsule^[76]. The rate of uneventful completion of the VCE examination after successful excretion of the patency capsule approximates 100%, even though excretion times may vary between patients^[58]. In cases of unsuccessful patency capsule procedure, the small bowel should be investigated by alternative diagnostic modalities such as cross sectional imaging (MR-E).

CONCLUSION

VCE possesses several important diagnostic advantages for IBD patients, mainly excellent visualization of the entire small bowel mucosa and excellent tolerability. The main challenge for further implementation of VCE in monitoring of IBD patients is an establishment of a validated quantitative score for assessment of mucosal healing and postoperative recurrence, that would allow routine utilization of this modality in both clinical practice and clinical trials. This could be especially important in CD, where outcomes in clinical trials are frequently assessed using surrogate markers (clinical scores, inflammatory markers) and evaluation of the mucosal healing limited to the colon and terminal ileum, that frequently does not reflect the inflammatory burden of the small bowel.

Small bowel lesions are frequently diagnosed in patients initially diagnosed with UC or after IPAA. The true clinical significance of these lesions, and whether they actually represent undiagnosed cases of CD is an important question that merits further clinical and translational studies.

Another important pitfall limiting the use of VCE for CD monitoring is the clinician's reluctance to perform VCE in these patients due to an exaggerated concern of retention. However, routine utilization of a patency capsule improves the safety of this procedure significantly, rendering the actual risk of retention extremely low.

However, patency capsule frequently results in additional costs.

Further technological enhancements in the future may potentially lead to a further expansion of the indications for capsule endoscopy in IBD (Table 5). These improvements may include a development of an externally operated capsule, that has already been attempted^[77,78]. An additional significant limitation of the capsule endoscopy is a lack of sampling ability, diminishing its usefulness for monitoring of neoplasms and colonic or small bowel dysplasia. In the future, additional technological features that are under development including tissue diagnosis capabilities, fluid aspiration, drug delivery and therapeutic (coagulation) capabilities may further increase the clinical utility of this modality^[79].

VCE is a very important tool for diagnosis of CD, and also has a potentially significant role in the therapeutic monitoring of these patients. Capsule endoscopy may also provide important clinical information for patients with IBDU, UC and pouchitis, with an excellent tolerability and safety profile. Indications for VCE in IBD are likely to increase in the future with further technological and clinical developments.

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Intestinal barrier in inflammatory bowel disease

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Abstract

A complex mucosal barrier protects as the first line of defense the surface of the healthy intestinal tract from adhesion and invasion by luminal microorganisms. In this review, we provide an overview about the major components of this protective system as for example an intact epithelium, the synthesis of various antimicrobial peptides (AMPs) and the formation of the mucus layer. We highlight the crucial importance of their correct functioning for the maintenance of a proper intestinal function and the prevention of dysbiosis and disease. Barrier disturbances including a defective production of AMPs, alterations in thickness or composition of the intestinal mucus layer, alterations of pattern-recognition receptors, defects in the process of autophagy as well as unresolved endoplasmic reticulum stress result in an inadequate host protection and are thought to play a crucial role in the pathogenesis of the inflammatory bowel diseases Crohn's disease and ulcerative colitis.

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Key words: Intestinal barrier; Antimicrobial peptide; Mucus layer; Inflammatory bowel disease; Crohn's dis-

ease; Ulcerative colitis; Goblet cell; Paneth cell

Core tip: An efficient intestinal mucosal barrier is critical for protection against invading microorganisms, therefore impairments in this system have serious adverse effects on health. In patients suffering from the inflammatory bowel diseases Crohn's disease and ulcerative colitis, the intestinal barrier function is compromised at different levels including, amongst others, a defective production of antimicrobial peptides, alterations of the mucus layer and defects in the process of autophagy. In this article, we outline important components of the healthy intestinal mucosal barrier and review their disturbances in inflammatory bowel disease.

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INTRODUCTION

Besides its most obvious function - digestion and nutrient absorption - the intestinal tract is challenged with another complex and difficult task. As the gut lumen harbors an enormous number of microorganisms, the host has to provide a peaceful coexistence with this diverse microbial community. The successful handling of this challenge includes on the one hand prevention of an inordinate immune response against commensals and on the other hand a proper detection and elimination of pathogenic microorganisms^[1-4]. The intestinal colonization increases from proximal to distal and reaches the enormous number of 10¹¹-10¹² organisms per gram of luminal content in the colon^[5,6]. To prevent a harmful adhesion and invasion of microorganisms, the intestinal mucosa is equipped with diverse specific and unspecific protective mechanisms that collectively build a complex

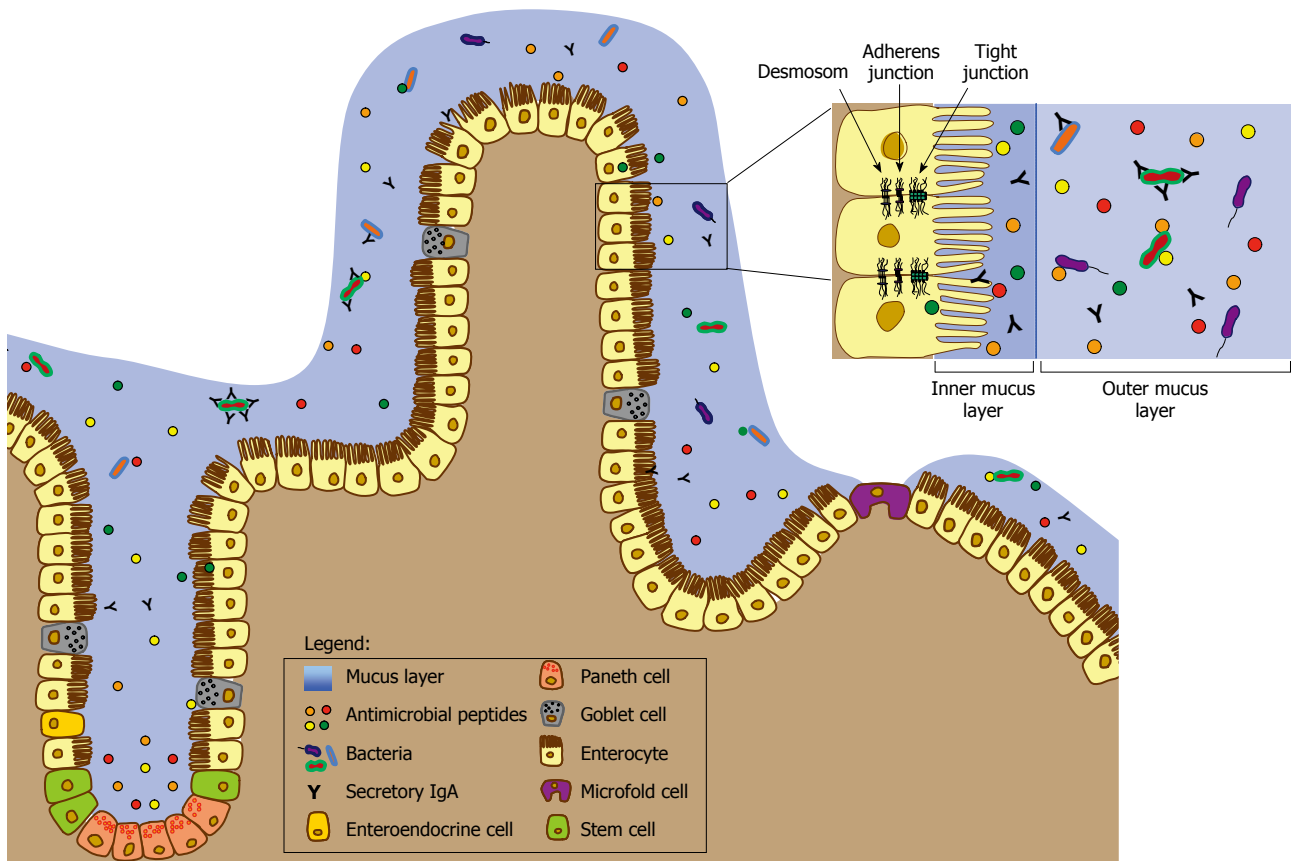


Figure 1 Defense mechanisms of the healthy intestinal mucosal barrier.

and effective mucosal barrier^[7-9] (Figure 1). Some important parts of this barrier concerning the innate immunity will be discussed in this article.

COMPONENTS OF THE INTESTINAL MUCOSAL BARRIER

The epithelium

The intestinal epithelium lines the luminal surface of the gut as a single layer of closely adhering cells and forms an efficient physical boundary between luminal contents and the body's interior^[2,4,10]. The appearance of the small intestine and the large intestine epithelium differs in that the surface area of the small intestine epithelium is highly increased by the formation of a large number of protrusions (folds, villi, microvilli) to accomplish the task of nutrient absorption. Situated between the villi there are glandular invaginations that are called crypts of Lieberkühn. In contrast, the epithelium of the colon has a significantly less enlarged and flat surface since it lacks the villi^[2,11].

The most abundant cell type of the intestinal epithelium is the absorptive enterocyte^[8,12]. Apart from enterocytes there are three different cell types with specific secretory functions, namely goblet cells, enteroendocrine cells and Paneth cells. Goblet cells are specialized in the secretion of mucus constituents and enteroendocrine cells secrete peptide hormones that are involved in cel-

lular trophism, tissue repair, angiogenesis, enterocyte differentiation and polarization along the crypt-villus axis^[3,11]. The third secretory cell-type of the intestinal epithelium, the Paneth cell, is named after the Austrian physiologist Joseph Paneth. Paneth cells contain a large number of secretory granules filled with large amounts of antimicrobial active substances like lysozyme, secretory group II A phospholipase A2, α -defensins and the C-type lectin regenerating islet-derived protein 3- α (also known as HIP/PAP, hepatointestinal pancreatic protein), of which the α -defensins are by far most abundant. Paneth cells are long-lived cells and normally are confined to the small intestine where they are localized at the bottom of the crypts of Lieberkühn^[1,13,14]. Furthermore, the intestinal epithelium contains microfold-cells that are part of the follicle-associated epithelium and responsible for the transport of luminal bacteria and antigens to subadjacent immune cells^[4,7].

The intestinal epithelium provides a selective permeable barrier as it allows the passage of water, electrolytes and dietary nutrients but prevents a detrimental invasion of foreign antigens, microorganisms and their toxins^[15]. In order to fulfill this challenge, intestinal epithelial cells are closely interconnected by different protein complexes comprising tight junctions, adherens junctions and desmosomes. These cell-cell connections are required to stabilize the mechanical cohesion of the cells, to define the border between the apical and the basolateral mem-

brane regions and are also essential for the regulation of the paracellular permeability. In all three types of adhesion complexes, the cell-cell contact is mediated by an interaction between the extracellular region of specific transmembrane proteins, additionally the intracellular region of these proteins is connected *via* adaptor proteins to the cytoskeleton^[11,15]. Four types of integral membrane proteins, namely occludin, the claudins, tricellulin and junctional adhesion molecules, are the main protein components of tight junctions^[16].

Epithelial homeostasis for an effective intestinal barrier is maintained by the balance of cell proliferation and epithelial apoptosis. Important regulators of this homeostasis are the transforming growth factor (TGF)- α , stimulating proliferation, and TGF- β inhibiting cell growth *via* signalling pathways. Also an essential factor for the renewing of the intestinal epithelium is Wnt^[17]. The canonical Wnt/ β -catenin pathway regulates epithelial proliferation and the non-canonical Wnt5a pathway is essential for the formation of new crypts^[18]. Fevr *et al*^[19] could show that in the Wnt/ β -catenin signalling a lack of β -catenin resulted in a differentiation of stem cells leading to the disturbance of the intestinal homeostasis.

With regard to the recognition of microbes and the initiation of appropriate immune responses of special importance are so called pattern-recognition receptors (PRRs) that are expressed by different cell types within the gastrointestinal tract including epithelial cells and immune cells. The two most investigated families of PRRs are the intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and the membrane bound toll-like receptors (TLRs). In general, PRRs detect microorganisms by conserved structural motifs that are characteristic for them, for example lipopolysaccharide, peptidoglycan, bacterial DNA and flagellin. Upon ligand binding, a signalling cascade is initiated that finally leads to the activation of signalling molecules like nuclear factor κ B and the expression of proinflammatory cytokines, chemokines or antimicrobial peptides^[4,8,20].

Antimicrobial peptides

Antimicrobial peptides (AMPs) are an integral part of the innate immunity. Their expression has been highly conserved during evolution as plants, insects, bacteria and vertebrates all make use of a variety of AMPs to protect themselves against microorganisms^[21,22]. Generally, classical AMPs are small cationic peptides with an amphipathic structure^[23-25]. The two best characterized families of AMPs in mammals are the defensins and the cathelicidins^[25].

Characteristic features of defensins are their low molecular weight, their cationicity and a specific fold rich in β -sheet structures^[26,27]. In humans, two defensin subfamilies, α - and β -defensins, are classified according to characteristic differences in their intramolecular disulfide bonding pattern^[28]. The members of both subfamilies are synthesized as pre-pro-peptide that need proteolytic processing to become the mature biologically active peptides

exhibiting a broad spectrum of antimicrobial activity^[27,29].

Of the six so far known α -defensins, two are of particular importance in the small intestine. They are named human defensin 5 and human defensin 6 (HD5 and HD6) and in the healthy intestinal tract they are synthesized in high amounts by Paneth cells in the small intestine^[30-32]. As Paneth cells are normally restricted to the small intestine, expression of Paneth cell α -defensins is absent in the healthy colon. However, in the case of inflammatory bowel disease (IBD), development of metaplastic Paneth cells and subsequent α -defensin expression in the large intestine may occur^[26,33]. The four remaining members of the α -defensin family are called human neutrophil peptide 1-4 according to their principal site of expression, namely neutrophilic granulocytes^[34]. There they are stored primarily as mature peptides in azurophilic granules but also in smaller amounts in their proform in specific granules^[29,35]. β -defensins are mainly of epithelial origin and have been identified in various epithelia throughout the human body including the intestinal tract^[36-39], the respiratory tract^[40,41], and the skin^[42,43]. Interestingly, environmental impacts seem to have an important influence on the functional activity of the human β -defensin 1 (HBD-1). The antimicrobial activity of this β -defensin depends to a large extent on its redox-status as reduction of disulfide bridges strongly enhanced its antimicrobial effect against the facultative pathogenic yeast *Candida albicans* as well as against anaerobic Gram-positive *Bifidobacteria* and *Lactobacilli*^[44].

Some defensins are produced constitutively and provide a continuous host-defense as the β -defensin family member HBD-1. Alternatively, their expression may be inducible by proinflammatory cytokines or in the presence of microorganisms and their products. This expression pattern is shown for example by the β -defensins HBD-2 and HBD-3^[21,45].

The only known human cathelicidin is named LL-37 and has a broad spectrum of antimicrobial activity. The peptide is synthesized as a proform (called hCAP-18, human 18 kDa cationic antimicrobial protein) from which it is released by proteolytic processing^[25,46,47]. However, studies on sweat and seminal plasma showed that a differential cleavage of hCAP-18/LL-37 is also possible, giving rise to alternative peptides with antimicrobial activity^[48-50].

Apart from these typical AMPs, antimicrobial properties have been attributed to several proteins with other classical functions. For example, microbicidal activities have been described for members of the families of histones and ribosomal proteins as well as for ubiquitin^[51-54].

Intestinal mucus layer

Another form of protection against invading microbes as well as other harmful insults from the intestinal lumen, including mechanical injuries or destructive enzymes, is provided by the intestinal mucus layer that coats the mucosa as a sticky gel. Apart from its protective function, mucus also has a lubricating capacity and is important to

keep the mucosal surface hydrated^[55,56]. It is composed of proteins, carbohydrates and lipids and also contains a very high degree of water^[57,58].

Essential for the formation of an intact and stable mucus layer are large glycoproteins belonging to the family of the secretory gel-forming mucins^[59,60]. The mucin MUC2 is the predominant member of this family in the intestinal tract where it is strongly secreted by goblet cells of the small and large intestine^[60,61]. By forming net-like multimeric complexes, MUC2 acts as the principal structural unit of the mucus layer. Colonic mucins carry a negative charge since their carbohydrates are substituted with numerous sulfate and sialic acid residues^[62,63]. These residues offer the mucins extra protection against bacterial attack and enzymatic degradation^[59,64-66].

In the large intestine, the mucus layer is divided into two distinct sectors with individual properties. The inner layer is densely packed, firmly attached to the epithelium and only removable by mechanical scraping, whereas the outer layer is not as tightly bound and can easily be aspirated with a micropipette. Interestingly, in the healthy colon the inner layer is sterile and efficiently prevents a direct contact of the epithelium with the luminal microbial community, whereas the outer layer is colonized by commensal bacteria. As studies on MUC2^{-/-} mice demonstrated, formation of an efficient mucus layer is not feasible in the absence of MUC2. In a study by Van der Sluis *et al.*^[67], MUC2^{-/-} mice developed spontaneous colitis and were more susceptible to dextran sulfate sodium (DSS) induced colitis. Moreover, Johansson *et al.*^[68] detected in MUC2^{-/-} mice bacteria in direct contact with the epithelium, deep down in the crypts and sometimes even inside epithelial cells, whereas in wild type mice the inner mucus layer was sterile and provided an efficient spatial separation of microbes and the epithelium.

Apart from MUC2, Fc-gamma binding protein (Fcgbp) has been identified as a mucus constituent with apparent structure-providing properties. Originally, the protein was named after its capability to bind to the Fc-part of IgG antibodies. Fcgbp-expression has been detected in the mucus granules of goblet cells and the protein has been identified in large intestinal mucus. There, it is covalently attached to MUC2 and is therefore supposed to act as a MUC2-cross linker that stabilizes the MUC2 network^[69-71]. Fcgbp in turn, has been identified as the disulfide linked partner of another protein-constituent of the mucus layer, the trefoil factor TFF3. It is mainly expressed in intestinal goblet cells and has been linked to mucosal protection and repair processes^[72].

Moreover, the amphiphilic phospholipid phosphatidylcholine (PC) has been identified as an important mucus constituent. The interactions between PC and mucins are most likely electrostatically owing to attraction between the negatively charged mucins and the positively charged molecule regions of PC. These interactions are thought to generate a spatial arrangement where the fatty acid chains of PC are oriented to the intestinal lumen generating a protective hydrophobic surface on top of

the mucus layer^[73,74].

However, since the mucus layer exhibits also antimicrobial properties, it acts not only as a physical barrier. The additional function of intestinal mucus as a chemical barrier was first indicated by a study of Meyer-Hofert *et al.*^[75] in which the antimicrobial properties of the murine small intestine were analyzed and a strong antimicrobial activity of mucus extracts against commensal and pathogenic bacteria was demonstrated. Moreover, various AMPs were identified in mucus extracts and, as the peptide spectrum resembled the spectrum of AMPs found in extracts from the whole small intestine as well as isolated crypts, it was assumed that epithelial AMPs are retained and thereby enriched in the murine small intestinal mucus layer. In another study, the antibacterial lectin RegIIIγ has been shown to be essential for the maintenance of a sterile zone of approximately 50 μm thickness above the murine small intestinal surface^[76]. This critical contribution of an antibacterial molecule to an effective spatial segregation of luminal bacteria and the small intestinal surface also points to an antibacterial capacity of the murine small intestinal mucus layer. To enlarge the available knowledge of mucus as an antimicrobial barrier, we recently performed detailed investigations of human large intestinal mucus concerning its antimicrobial properties and revealed a high antimicrobial activity of mucus protein extracts against Gram-positive and Gram-negative bacteria as well as against the yeast *Candida albicans*. Several peptides and proteins with reported antimicrobial functions including defensins, the cathelicidin LL-37, ubiquitin and members of the family of histones could be identified in rectal mucus extracts^[77]. Interestingly, several of these peptides were already identified in non-inflamed human colonic tissue in two previous studies^[78,79], supporting the hypothesis that epithelial AMPs are retained in the mucus layer. Moreover, we demonstrated a binding of AMPs to mucus and mucins and also showed that this binding does not abolish the peptides functional activity^[77]. As large intestinal mucins are negatively and cationic AMPs positively charged, binding of AMPs to intestinal mucus is most likely due to electrostatic interactions between these oppositely charged molecules^[80,81].

Besides antimicrobial peptides, mucus also contains secretory IgA that is translocated across the epithelium in large amounts and helps to prevent an invasion of luminal microorganisms^[55,82].

INTESTINAL BARRIER IN IBD

Defects in the intestinal epithelial barrier function are a characteristic feature of IBD. The perturbations can affect different levels of the protective mechanisms, including alterations of PRRs, disturbed AMP production, a defective mucus layer, alterations in the process of autophagy or an increased epithelial barrier permeability, and cause an inadequate protection against microbial adherence and invasion. The resulting imbalance between

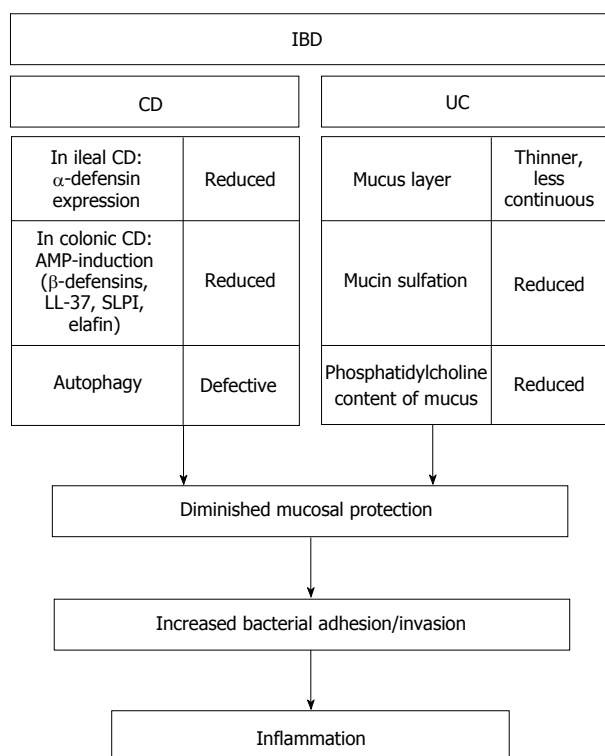


Figure 2 Defects of the intestinal mucosal barrier in inflammatory bowel disease and their consequences. IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

the microbes and the protective host-defense mechanisms allow more bacteria to come in direct contact with the epithelium and mucosal immune mechanisms. Consequently, compensatory immune reactions are excessively triggered, a process that is thought to finally result in chronic intestinal inflammation (Figure 2).

As IBD affects predominantly the most heavily colonized gut segments, namely the distal ileum and colon, an involvement of the microbial flora in disease pathogenesis is obvious^[5]. This is also indicated by studies showing that postoperative recurrence of Crohn's disease (CD) is triggered by luminal contents^[83,84]. Moreover, several animal models revealed the essential contribution of microbes to the development of inflammation^[85,86]. Yet, despite extensive research, no specific IBD-causing pathogen could be identified until now^[87,88]. Instead, the intestinal microbial community as a whole appears to be crucially involved in disease pathogenesis^[89]. Studies showing a marked increase in surface associated bacteria and to some extent also intracellular bacteria in patients with IBD confirm this assumption^[90-92]. This suggests a deficiency in the protective mechanisms that effectively keep away microbes from the epithelial surface in the healthy intestine. In the following sections, important aspects of barrier perturbations in IBD will be discussed in more detail.

Pattern-recognition receptors and antimicrobial peptides

In 2001, different groups identified independently the NLR-family member nucleotide-binding oligomerization

domain-containing protein 2/Caspase recruitment domain-containing protein 15 (NOD2/CARD15) as a susceptibility gene for CD^[93,94]. There are three common genetic variants associated with CD, two missense mutations and one insertion frameshift mutation that leads to a truncation of the mature NOD2 protein^[4,20]. As Kobayashi *et al*^[95] showed in a study on NOD2^{-/-} mice, NOD2 plays a pivotal role in the intestinal host-defense. The NOD2-deficient mice were highly susceptible to intragastric challenge with *Listeria monocytogenes* but not to intraperitoneal or intravenous infection and displayed reduced levels of a subset of Paneth cell α -defensins. Furthermore, the important role of NOD2 in intestinal homeostasis was underscored by the observation that NOD2 is crucially involved in the regulation of the intestinal flora. Alterations in the composition and quantity of the commensal microbiota were observed in NOD2-deficient mice^[96]. Interestingly, CD patients homozygous for the NOD2 variant SNP13 displayed similar changes that were characterized by a significantly increased load of Bacteroidetes and Firmicutes in their terminal ilea^[96]. Moreover, Petnicki-Ocwieja *et al*^[97] detected increased amounts of commensal bacteria in the terminal ilea, a diminished capability to prevent *de novo* colonization of the opportunistic pathogen *Helicobacter hepaticus* and a reduced antimicrobial activity of crypt secretions in NOD2-deficient mice. Notably, an interrelation of NOD2, antimicrobial peptide expression and intestinal defense in humans was emphasized by a study showing reduced levels of Paneth cell α -defensins in patients with ileal CD that was accompanied by a reduced antibacterial activity of protein extracts from ileal mucosa. Interestingly, the defensin reduction was especially pronounced in patients carrying the mutation NOD2-SNP13^[98]. These findings highlight the important role of a diminished antibacterial host-defense as a primary factor in disease pathogenesis.

Recently, a second member of the NLR-family, namely NLR family, pyrin domain containing 3 (NLRP3), has been implicated in the pathogenesis of IBD. NLRP3 is as a constituent of the inflammasome involved in the activation of caspase-1 and the subsequent maturation of interleukin-1 β and interleukin-18^[99,100]. Villani *et al*^[101] reported an association between SNPs (Single nucleotide polymorphisms) located in a predicted regulatory region downstream of NLRP3 and an increased risk to develop CD and Schoultz *et al*^[102] suggested a role for combined polymorphisms in CARD8 and NLRP3 in the development of CD in men but an implication of the NLRP3-region in CD-susceptibility was not confirmed in a recent study^[103]. Thus, the available data in this context are inconsistent and further studies are required.

However, studies on NLRP3^{-/-} mice showed that NLRP3 plays a central role in the regulation of intestinal homeostasis and prevention of colitis. Zaki *et al*^[104] studied NLRP3^{-/-} mice and showed that these mice were significantly more susceptible to DSS-induced colitis compared to wild-type mice. A clearly increased susceptibility of NLRP3^{-/-} mice to experimental colitis was also report-

ed by Hirota *et al.*^[99] whereas, in contrast to these studies, Bauer *et al.*^[105] found that NLRP3-deficient mice were significantly protected from colitis in the DSS-colitis model. Interestingly, in the study of Hirota *et al.*^[99] the NLRP3^{-/-} mice displayed also an altered colonic β -defensin expression, a reduced antimicrobial activity of colonic crypt secretions and an intestinal dysbiosis. These results are of special interest with regard to the pathogenesis of IBD as a dysregulated β -defensin expression has been linked to the development of colonic CD. Macroscopically and histologically non-inflamed tissue samples of CD-patients with colonic involvement displayed reduced mRNA-levels of the constitutively expressed β -defensin HBD-1^[106]. Moreover, defects in the expression of the inducible β -defensin HBD-2 were reported. In ulcerative colitis (UC)-patients, the expression of this β -defensin is strongly induced in the case of inflammation but this induction is diminished in patients with colonic CD^[39] and the expression of two other β -defensins, HBD-3 and HBD-4^[37,39] as well as the cathelicidin LL-37^[107] and the antimicrobially active protease inhibitors SLPI and elafin^[108] seems to follow a similar pattern. The functional relevance of these findings was pointed out by a study showing a reduced antimicrobial activity of colonic tissue protein extracts in colonic CD patients^[109].

Moreover, polymorphisms in the genes of several members of the TLR-family have also been associated with IBD^[11] and especially extensively investigated was the influence of TLR4-polyomorphisms on the risk to develop CD. Whereas some studies showed a correlation between TLR4 genetic variants and CD^[110-112], in other studies no such association was found^[113-115]. Thus, the available data in this regard are contradictory and no definite conclusion can be drawn to date.

PANETH CELLS AND GOBLET CELLS

Wnt-pathway

As NOD2 and α -defensins are both associated with small intestinal Paneth cells, it was apparent that defects in Paneth cell biology play a crucial role in small intestinal CD. Thus, further studies were conducted to characterize the role of Paneth cell-dysfunction and diminished antimicrobial host-defense in CD in more detail and it was shown that the Wnt-pathway transcription factor T-cell specific transcription factor 4 (TCF-4, also known as TCF7L2, transcription factor 7-like 2) is critically involved in this context. Patients with ileal CD displayed, independent of the degree of inflammation, a reduction in TCF-4 mRNA-expression and the TCF-4-mRNA-levels correlated highly with the mRNA-levels of the Paneth cell α -defensins. Importantly, the association between α -defensins and TCF-4 was independent of the NOD2-genotype. Additionally, a causal link between reduced TCF-4- and reduced α -defensin-levels was demonstrated by a TCF-4-knock out mouse model in which TCF-4-heterozygous mice displayed a significantly

reduced α -defensin expression that was associated with a reduced antibacterial activity of small intestinal tissue extracts^[116]. Moreover, in a later study an association between a sequence polymorphism in the TCF-4 putative promoter region and a higher susceptibility to develop small intestinal CD was reported^[117]. Recently, it was uncovered that another central factor of the Wnt-pathway, the co-receptor low density lipoprotein receptor-related protein 6 (LRP6), is required for proper antimicrobial Paneth cell function and is involved in the development of CD. More specifically, a functional variant in the Wnt co-receptor (rs2302685; Ile1062Val) has been associated with early onset ileal CD and, importantly, patients carrying this variant were shown to have particularly low Paneth cell α -defensin-levels. Moreover, the LRP6-mRNA-levels were generally reduced in ileal CD independently of the genotype^[118]. In conclusion, the link between genetic variants in crucial components of the Wnt-pathway, diminished α -defensin levels and ileal CD indicate that the diminished Paneth cell antimicrobial function is a primary pathogenic factor in CD. Moreover, since the Wnt-pathway does not only directly regulate α -defensin expression but also influences Paneth cell maturation, these data suggest that an impaired Paneth cell differentiation may be involved in CD pathogenesis^[113,119].

ER-stress and the unfolded protein response

In recent years, unresolved ER-stress and the unfolded protein response (UPR) have repeatedly been implicated in the pathogenesis of CD as well as UC. A first indication of a relation between ER-stress and intestinal inflammation was provided by a study from 2001 that revealed an increased susceptibility to DSS-induced colitis in mice deficient in the ER-stress transducer IRE1 β ^[120]. In a more recent study, deletion of another key component of the ER-stress response, the transcription factor X-box-binding protein 1 (XBP1), in the intestinal epithelium of mice lead to increased ER-stress, spontaneous small intestinal inflammation and also increased sensitivity towards induced colitis. Interestingly, Paneth cells were absent in these mice and the number of goblet cells in the small intestine was also reduced. As Paneth cells normally secrete large amounts of antimicrobial molecules, the Paneth cell depletion resulted in a reduced antibacterial activity of crypt supernatants as well as an increased susceptibility to oral infection with *Listeria monocytogenes*. Importantly, a possible link between XBP1 and human disease was provided by the detection of an association between genetic variants of XBP1 and IBD^[121]. Moreover, another clear indication that the process of ER-stress may be implicated in IBD pathogenesis gave the detection of an overexpression of the ER-localized stress response chaperone Gp96 on the apical surface of ileal epithelial cells in patients with CD. This chaperone is used as a receptor for adherent-invasive *Escherichia coli* (*E. coli*) invasion *via* outer membrane vesicles (OMVs) rich in the outer membrane protein OmpA^[122]. These are

especially interesting findings with regard to the older observation that adherent-invasive *E. coli* strains are highly associated with the ileal mucosa in CD^[123-125].

Furthermore, particularly noteworthy in the context of ER-stress and IBD pathogenesis are several studies that provide a link between ER-stress, defective mucus production and susceptibility to intestinal inflammation. Heazlewood *et al.*^[126] generated and characterized two mice-strains, called Winnie and Eeyore, each with a single missense mutation in the *MUC2* gene. These mice developed spontaneous intestinal inflammation and showed a phenotype reminiscent of human UC. Moreover, they were more susceptible to environmentally induced colitis and showed profound changes in the process of MUC2 synthesis and assembly. This went along with an abnormal mucin distribution in goblet cells - the goblet cell theca were smaller and reduced in number and a cytoplasmic accumulation of the non-O-glycosylated MUC2-precursor in vacuolar structures was detectable. Importantly, goblet cells showed ultrastructural and biochemical evidence of ER-stress and activation of the UPR. Furthermore, in tissue samples of UC patients a cytoplasmic MUC2-precursor accumulation was also detectable and this coincided with signs of protein misfolding and ER-stress. From these findings the authors concluded that ER-stress related changes in mucin production could be crucially involved in the pathogenesis of UC.

Also linked to mucus production, the ER-stress response and prevention of intestinal inflammation is anterior gradient homolog 2 (AGR2), a member of the family of protein disulfide isomerases that is present in the ER of intestinal secretory epithelial cells. Recently, AGR2 has been shown to play an essential role in intestinal mucus production and in this context, a direct association of AGR2 and MUC2 *via* the formation of mixed disulfide bonds between a cysteine in the thioredoxin-like domain of AGR2 and cysteines in the amino- and carboxyl-terminal portions of MUC2 are thought to be crucial. The importance of AGR2 for mucus production was demonstrated in AGR2^{-/-} mice that lacked intestinal mucus and MUC2 protein and showed increased susceptibility to DSS-induced colitis. Although the mice developed no overt spontaneous inflammation, they displayed an increase in the expression of several proinflammatory cytokines and in the presence of intestinal mast cells^[127]. In a more recent study, AGR2^{-/-} mice generated by Zhao *et al.*^[128] displayed severe spontaneous terminal ileitis and colitis. Moreover, the mice lacked morphologically normal goblet cells and showed decreased MUC2 protein levels. Interestingly, the intestinal disturbances were not restricted to goblet cells as severe Paneth cell abnormalities were also detectable including a dramatic expansion of the Paneth cell compartment and an abnormal Paneth cell localization. As the Paneth cell expansion preceded the occurrence of inflammation it was not a secondary phenomenon resulting from inflammatory processes. Whereas Park *et al.*^[127] reported only a modest evidence

for activation of the ER-stress response, Zhao *et al.*^[128] detected clearly elevated levels of intestinal ER-stress. Albeit the definite cause is to date not clear, the discrepancies between the studies of Park *et al.*^[127] and Zhao *et al.*^[128] were attributed to differences in the genetic background and experimental design as well as to diverging housing conditions. In conclusion, the mentioned AGR2^{-/-} mouse models underline the importance of AGR2 in the intestinal goblet cell- as well as Paneth cell-homeostasis and the ER-stress response and are particularly interesting since genetic variants of AGR2 and decreased AGR2-mRNA-levels have been linked to IBD^[129].

Autophagy

Autophagy is a degradation process important for cellular homeostasis that allows the cell to recycle cellular components by delivering organelles and cytosolic macromolecules to lysosomes. The process is activated in response to situations of cellular stress like starvation and growth factor deprivation. Moreover, the autophagy machinery is also important in the antibacterial host-defense as it is involved in the degradation of invading bacteria^[130-132].

Recently, a defect in the process of autophagy has been linked to the pathogenesis of CD as a polymorphism in the gene encoding the central autophagy protein autophagy-related 16-like 1 (ATG16L1) has been associated with an increased risk to develop the disease^[133]. Moreover, a second autophagy-related protein, immunity-related GTPase family M protein (IRGM), has been related to CD^[131,134,135]. In subsequent studies, the role of ATG16L1 in CD-pathogenesis has been investigated in more detail and it was reported that Paneth cells of ATG16L1-hypomorphic mice displayed abnormalities in the granule exocytosis pathway. Importantly, homozygous carriers of the disease associated risk allele of ATG16L1 among CD patients exhibited similar changes in Paneth cell granules^[136]. Additionally, Kuballa *et al.*^[130] demonstrated in cell culture experiments that the CD-associated ATG16L1 coding variant (ATG16L1*300A) is linked to a defective anti-Salmonella autophagy. Furthermore, a link between the process of autophagy and CD was provided by a study on paediatric CD patients that revealed an activation of autophagy in Paneth cells that occurred independently of inflammation or disease-associated variants in ATG16L1 or IRGM and went along with a significant reduction in the number of secretory granules and signs of crinophagy^[137]. Remarkably, in a recent study the intracellular PRRs NOD1 and NOD2 have been shown to be involved in the induction of autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. As the CD-associated NOD2 mutant L1007fsinsC was defective in this process, these findings provide a mechanistic link between two of the most important genes associated with CD, NOD2 and ATG16L1, and highlight the relevance of defects in the process of bacterial sensing and elimination in the disease pathogenesis^[138].

ALTERATIONS OF THE MUCUS LAYER

Variations of the intestinal mucus layer concerning its thickness, continuity and composition as well as the mucin-structure have been reported in IBD patients, especially in UC. These changes are thought to adversely affect the protective properties of the gel layer and consequently might cause an increased vulnerability of the epithelium to bacterial invasion.

The mucus layer has been shown to be thinner and less continuous in UC patients^[139], its phosphatidylcholine concentration is significantly reduced^[73] and, in some older studies, a selective reduction of a specific mucin glycoprotein fraction in the colonic mucosa has been reported^[140,141]. However, as the latter results could not be reproduced in a subsequent investigation^[142], they were interpreted as an effect of the experimental design and were thought to rather reflect the general mucus depletion characteristic for UC patients^[142,143]. Furthermore, an altered mucin glycosylation was detected in UC patients - the glycans are shorter, have a generally less complex structure^[144,145] and their sulfation is reduced^[64,146]. As the sulfates confer negative charge to the mucins and enhance the resistance of the glycans against enzymatic degradation, a reduced sulfation could result in an increased vulnerability of the mucins to bacterial enzymatic degradation^[64,66,146] and, since positively charged AMPs are electrostatically bound by mucins^[80,81], might cause a less effective retention of AMPs in the mucus layer. Whether the reported changes are primary events in disease pathogenesis or are secondary effects resulting from inflammation is currently under discussion. Whereas some studies indicated that mucus or mucin alterations occur only in active UC^[144,147], other studies gave evidence of primary alterations as they revealed an ethnic and genetic impact^[141,148-150]. Recently, mucus depletion in UC has been linked to a defect in goblet cell differentiation as the significant induction of the goblet cell differentiation factors *Hath1* and *KLF4* seen in CD patients in inflammation was not detectable in UC patients and, moreover, a reduced number of mature goblet cells in the upper third of the crypts in sigma tissue of patients with active UC was observed^[151].

EPITHELIAL PERMEABILITY

Already in the early 1980s, increased intestinal permeability has been reported in children with active small-bowel CD^[152] and, more recently, increased permeability has also been described in UC patients^[153,154]. Several studies observed increased permeability not only in CD patients but also in a subset of their first-degree relatives, leading to the assumption that increased permeability may be a primary factor in disease pathogenesis preceding the development of inflammation^[155-157]. Interestingly, in a recent study an association between a high mucosal permeability in healthy first-degree relatives of CD patients with the presence of a *CARD15*

3020insC-mutation has been observed^[158]. Moreover, increased permeability has been linked to a higher risk of disease relapse in CD patients^[159,160], suggesting that the permeability increase precedes clinically overt inflammation. However, in other studies a permeability increase was also detected in a proportion of spouses of CD patients^[161-163], an observation that indicates an impact of environmental factors on permeability alterations. To date, it is still under debate whether the permeability changes are a primary event in the disease development or a secondary effect triggered by inflammation. The perturbations of intestinal permeability are assigned to different molecular mechanisms including tight junction abnormalities^[8,15,164]. For example, in a recent study a reduced number of tight junction strands and an increased number of strand breaks were observed in CD patients^[165]. Moreover, an increased expression of the pore-forming claudin 2 and a decreased expression of occluding and the sealing claudins 5 and 8 was detectable. These changes were observed in patients with mild to moderately active CD but were absent from patients in remission indicating that they were a rather secondary phenomenon occurring as a consequence of inflammation^[165]. In UC claudin 2 was also upregulated, whereas occludin and the claudins 1 and 4 were diminished^[166].

Additionally epithelial apoptosis also results in an increased epithelial permeability.

Several studies showed that in inflammatory bowel disease apoptosis is upregulated. Sipos *et al.*^[167] found the rates of epithelial apoptosis determined by the histological activity of inflammation in UC. IL-13 triggers an increased epithelial apoptosis rate that leads to microerosions^[166] in an early stage of the disease. In contrast, in active CD apoptosis is also upregulated^[166,168], but microerosions are only induced in advanced disease.

Using confocal laser endomicroscopy, Goetz *et al.*^[169] found a higher density of epithelial gaps in the mucosa of patients with CD. In a mouse model TNF led to an elevated epithelial cell shedding and increased the gap density^[170].

In conclusion, epithelial permeability perturbations occur in IBD patients but additional investigations are required to further increase the knowledge about the underlying molecular mechanisms as well as to clarify whether these changes play a primary role in disease pathogenesis or whether they are a secondary effect in response to inflammation. It is important to note that carbohydrates such as lactulose and mannitol, the low molecular weight polyethylene glycol PEG 400 or ⁵¹Cr-labelled ethylenediaminetetraacetic acid were used as probe molecules to measure the permeability of the intestinal wall in the mentioned studies. As increased permeability for these substances does not give information on epithelial permeability for bacteria and bacterial products, it is not possible to deduce that the described permeability changes are associated with a defective protection of the mucosa against luminal microorganisms and an increased exposure of mucosal immune mechanisms to microbial

antigens.

CONCLUSION

The healthy intestinal mucosa is efficiently protected against detrimental influences from the gut lumen by a complex and multilayered defense barrier. In recent years, it became apparent that the intestinal barrier is compromised at different levels in IBD patients. These barrier disturbances include alterations of the mucus layer, a defective AMP production, unresolved ER-stress, defects in the process of autophagy as well as an increased epithelial permeability and result in an inadequate protection of the epithelium against adherence and invasion of luminal bacteria. Consequently, abnormal high numbers of microorganisms are allowed to come in direct contact with the hosts immune mechanisms and this is thought to initiate a process finally resulting in chronic intestinal inflammation.

Future studies are required to further increase our understanding of the complex mechanisms underlying the pathogenesis of IBD, to clarify their complex interplay and to enable the development of new therapeutic approaches for the treatment of IBD.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Magnetic resonance imaging in children and adolescents with chronic inflammatory bowel disease

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Abstract

Inflammatory bowel diseases (IBD) represent challenges, both from a diagnostic, and therapeutic point of view. Deep-seated anatomic structures are difficult to assess by ultrasound technique alone. As radiation-free alternative cross-sectional imaging method, magnetic resonance imaging of the intestinal structures is costly and time-consuming. Examination of pediatric patients imply additional considerations: reduction of body motions in younger children and consideration of the most appropriate preparation, and examination technique. The demanding Sellink technique is the only means for appropriately distending the lesser intestine in order to detect small bowel strictures. Oral intake of contrast medium (CM) alone shows its limitations regarding distensibility. The need for intravenous contrast media application needs to be considered, too. Active

inflammation of both intestinal wall, and mesentery can be demonstrated accurately. Nevertheless, viable alternatives to CM application is desirable, considering non-negligible adverse reactions. Recent data suggest diffusion weighted imaging might fill this diagnostic gap. Irrespective of sequence technique chosen, bowel movement remains a major obstacle. Antispasmodics in their function as smooth muscle relaxants help in improving image quality, however, their use in children might be off-label. Optimal preparation for the examination and appropriate imaging technique allow for diagnosing typical patterns of changes in IBD, such as bowel wall thickening, ulcers, mural stratification, strictures, creeping fat, and comb sign, and lymphadenopathy. The article gives a detailed overview of current significance of magnetic resonance imaging pediatric patients suffering from IBD, considering indications, limitations, and safety aspects.

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Key words: Children; Adolescents; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Magnetic resonance imaging; Enterography

Core tip: Diagnosis of chronic inflammatory bowel disease (IBD) is partially based on subsequent imaging. Magnetic resonance imaging (MRI) of the gastrointestinal tract (GIT) is established in adults for diagnosing IBD. In children and adolescents MRI is not routinely used up to now. This manuscript presents the commonly used magnetic resonance sequences for the evaluation of the GIT in children and adolescents. Techniques to obtain optimal bowel distension by oral intake or by using a nasally placed tube are described. Typical findings of intestinal and mesentery pathology in children suffering from IBD are shown.

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INTRODUCTION

Population-based studies suggest that inflammatory bowel disease (IBD) is unevenly distributed throughout the world with the highest incidence rate occurring in the Western world. However, emerging data have demonstrated a rising trend of IBD in Eastern countries^[1]. In approximately 20%-30% of all affected patients with IBD the disease manifests in childhood^[2]. Studies have shown a prevalence of as high as 16.6 per 100000 in the pediatric population^[3]. Chronic IBD, namely Crohn's disease (CD) and ulcerative colitis (UC) seem to develop as a result of dysregulation of the immune response to normal gut flora in a genetically susceptible host^[4] (Table 1). There are further reasons, like microvascular, infectious, and environmental co-factors to consider^[5,6]. Moreover changes in lifestyle and diet, migration-related changes in genetic susceptibility, urbanization, and environmental changes may be relevant cofactors. Epidemiologic data indicate an increase in IBD in children. While CD and UC occur with equal distribution in adults, there are three new CD cases for each new UC case in pediatric age groups^[7]. There are patients who, however, can't be classified as CD or UC. They are termed inflammatory bowel disease-undeterminate (IBD-U). In the pediatric population a greater number of IBD are labeled as IBD-U compared to adults. In adult IBD there is an equal ratio of male to female patients, or perhaps more women affected by the disease. In contrast, prepubertal males seem to be more affected than females, with a male predominance of 1.5:1^[8]. Rarely, UC can even be found in young children and infants. In children an overlap of clinical findings can be observed, making differentiation of both entities in the pediatric patient difficult. IBD in children often relapses, making repeated imaging necessary.

CD can affect any part of the gastrointestinal (GI) tract, however, in children the distribution tends to be more proximal. The terminal ileum is the most common site of CD; however about 60% of pediatric patients show ileocolonic involvement, whereas 20%-30% have isolated colonic disease^[1]. Transmural spread is a distinguishing feature of CD. Up to one third of patients will develop a perianal fistula or abscess at some point in their disease course. Skip lesions, fibrofatty proliferations, and mesenteric lymphadenopathy are common. In the case of chronification, fibrosis of the bowel wall may occur, resulting in stenotic bowel loops.

UC is mostly localized to the colon with the rectum affected primarily. Even if pancolitis is the most common presentation in childhood other features exist^[9]. UC is a chronic inflammation involving exclusively the

mucosa of the colon. Diarrhea and rectal bleeding is seen commonly in UC (50%-90% of cases)^[10]. Contrary, perianal or perirectal disease is not a feature of UC.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Diagnosis of IBD is based on clinical presentation, laboratory and endoscopic assessments, histology and subsequent imaging^[11,12]. Proper classification is necessary in order to determine the ideal treatment in children with chronic disease. The Montreal classification was adapted to the pediatric population^[13]. In a child with bloody diarrhea a bacterial infection needs to be excluded first. Further differential diagnosis includes vasculitis, ischemic colitis, and hemolytic uremic syndrome. Bleeding without diarrhea is possible in children with fissure, vascular malformations, coagulation disorders, polyps, or Meckel diverticulum. Etiologies that can mimic IBD include appendicitis. Moreover, food allergies like cow's milk protein allergy can result in GI bleeding as early as in infancy. Laboratory studies can help to identify a child suspected to suffer from IBD and to monitor the course of the disease. Diagnostic imaging is necessary to describe the extension and grading of the inflammation. In children, ultrasound can be used with high success. The use of high frequency transducers (7-14 MHz) allows a detailed assessment of the whole bowel. Graded compression allows for displacement of adjacent bowel loops and interfering intraluminal gas. Color Doppler can be used to assess inflammation by demonstrating increased perfusion and vascularization. Significant correlations could be shown by considering bowel wall thickness, vascular pattern, and disease activity. Ultrasound can be used to appreciate extraintestinal findings (lymph node enlargement, abscesses), and to describe the surrounding mesenteric tissue. On the other side, ultrasound can fail to detect IBD in cases of superficial disease or if there are artifacts (*e.g.*, bowel gas). Also, ultrasound is an operator dependent modality; experience is necessary in order to make the correct diagnosis. Plain radiography is reserved for acute diseases. Findings are nonspecific and can include large bowel dilation, and small bowel distension. In the acute abdomen, the toxic megacolon or obstruction can be suspected. These cases have to be followed in order to detect possible bowel perforation. Contrast enema can be helpful for the evaluation of the extension of the disease, moreover, it is helpful to rule out or prove stenosis. Upper gastrointestinal series with small bowel barium follow through (SBFT) has been the cornerstone of small bowel imaging in the past^[14]. The more sensitive enteroclysis was considered the gold standard (so-called Sellink technique), but it is not easy to perform, especially in younger children and infants. It requires the insertion of a naso-jejunal tube, for that it is poorly tolerated by children. It comes with a considerable higher radiation dose compared to SBFT^[15]. Both methods suffer from one further major disadvantage: its

Table 1 Inflammatory bowel disease in children

	Crohn's disease	Ulcerative colitis
Etiology	Unknown (hereditary, immune response to gut flora)	
Incidence		
Site	Terminal ileum/SB/entire GIT	Colon/backwash ileitis
Pattern	Skip lesions, transmural, deep ulcerations	Diffuse mucosal
Acute complication	Obstruction, toxicity, hemorrhage, perforation	
Chronic complication	Stenosis, fistula, fissures, abscess, GIT cancer, extraintestinal manifestation	Extraintestinal manifestation, colorectal cancer

SB: Small bowel; GIT: Gastrointestinal tract; PSC: Primary sclerosing cholangitis.

very limited information regarding the extraluminal mesenteric extension of the disease. Computed tomography (CT) as a cross-sectional imaging method overcomes this and may be helpful in the acute situation, for planning surgery, especially in the evaluation of stricture or obstruction. Again, CT enteroclysis is poorly tolerated in children^[16]. The major disadvantage of CT is the large amount of radiation exposure^[17]. So, especially in children, techniques using ionizing radiation should be avoided if possible^[18]. Leucocyte scintigraphy using white blood cells labeled with technetium 99m, and also positron emission tomography may be indicative in IBD, however, these techniques are not usually used. Because IBD is a diagnosis which should be proven by endoscopy with biopsy and histologic assessment, they are part of the diagnostic workflow. Endoscopy is the mainstay for evaluation, but provides limited access to the small bowel (terminal ileum and duodenum) and can be more limited in the presence of severe disease or strictures. Capsule endoscopy can overcome parts of these limitations, but has limitations as well especially in stricturing disease. Recent advances in magnetic resonance imaging (MRI) have the potential to emerge as useful imaging technique providing assessment of the whole gastrointestinal tract and to assess extra-intestinal involvement without ionizing radiation.

In adults MRI of the GI tract is now an established method when diagnosing chronic inflammatory bowel disease. In children and adolescents MRI is not routinely used, but some guidelines define MR as first line imaging modality in the investigation of the GI tract in suspected chronic inflammatory bowel disease. Especially the lack of radiation burden would favor the use of MRI. Further advantages include the superior soft tissue contrast and multiplanar imaging. Improvements in MR hardware include the availability of fast breath-hold sequences, decreased scan time and increased spatial resolution.

But, there are some difficulties of the technique in infants that limit the use of MRI. Children at school age have no problems with undergoing MR study, but the younger ones need to be handled gently because of reduced compliance. Numerous articles have been published on this topic discussing the complexity and also the financial impact on management. In the literature only few scientific reports and reviews on MRI of the GI tract in children can be found^[19]. The purpose of this article is to present the commonly used MR sequences in

children for the evaluation of the GI tract in the diagnosis and follow-up of IBD.

MR IMAGING OF THE BOWEL

Currently, there is no approved standard protocol for the evaluation of IBD using MRI. Applying a standard protocol without bowel distension is not helpful and results in false negative findings. The use of spasmolytics such as n-butylscopolamine (hyoscine) (0.5 mg/kg, up to 20 mg)^[20] or glucagon^[21] might meet the criteria of off-label use in some countries. Intravenous application of spasmolytics is given immediately before the start of the MR scans. A further bolus can be administered immediately before contrast media application. Metoclopramid (off-label in some countries) (0.15 mg/kg) can be given in the case of nausea. The cleansing preparation of the small bowel consists of a low-residue diet starting 3 d prior to the examination (no milk products, ample fluids, non-carbohydrate in order to avoid the generation of gas).

The cardinal principle to obtain diagnostic images is an optimal bowel distension of the bowel lumen. The loops need to be distended in order to detect changes in luminal diameter, which can be observed in cases with relative bowel stenosis. However, there is no consensus about the technique for optimal small-bowel distension^[22]. Commonly used agents include methylcellulose, locust-bean gum, psyllium, and mannitol. There are very different protocols in the literature, ranging from oral application before entering the MR cabin (hydro MRI) to the MR enteroclysis procedure (MR Sellink) using application of methylcellulose while in the MR scanner *via* a nasojejunal tube.

Oral intake of endoluminal agents is mandatory. Some studies have shown, that oral intake is sufficient and increases the patient's compliance (which is necessary especially in follow-up studies)^[23]. Unfortunately, sometimes the filling is inadequate and the distal/terminal ileum is not sufficiently distended (Figure 1). The most accurate results can be achieved with the MR enteroclysis technique^[24]. Reports in the literature describe numerous different oral contrast media suitable for use, including plain water, mannitol, sorbitol, iron oxide, and barium sulfate, ispaghula husk, locust bean gum, planta ovate, and pineapple juice^[25]. Based on their signal intensity in T1 weighted (T1w) and in T2 weighted (T2w) imaging, these contrast substances are divided

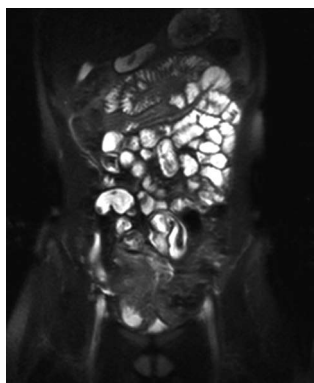


Figure 1 Hydro-magnetic resonance imaging using mannitol orally. Sometimes the filling result is inadequate and the distension of the bowel is insufficient.

into so-called positive (hyperintense in T1w and in T2w sequences) and negative (hypointense in T1w and in T2w sequences) contrast media. Some authors use a combination of barium sulfate and sorbitol as described by Sauer *et al.*^[26] who administered 450 cm³ of 0.1% barium sulfate with sorbitol 90 min before imaging and a second amount of 450 cm³ 30 min before imaging^[1]. Dagia *et al.*^[27] administered sorbitol orally (1000-1500 mL) as enteral contrast agent by adding 15 mL of sorbitol to 500 mL of water. Application started 60 min before the scan at a rate of 250-300 mL/15 min. If distension of the proximal small bowel was inadequate on initial MR acquisition (luminal diameter < 3 cm), another 250-300 mL of sorbitol was given^[27]. Others use a suspension of polyethylene glycol (PEG; Klean-Prep®, Helsinn Birex Pharmaceuticals Ltd.) one hour before the investigation (1500 mL) after an overnight fasting state^[20]. To render these mixtures more palatable, small amounts of orange flavoring may be added^[3]. Mannitol and other hypertonic contrast agents may have side-effects, such as excessive diarrhea, which is an important issue, especially in children. The influence of the osmolarity for small bowel distension could be shown in a study by Ajaj *et al.*^[25] who compared a water solution combined with 2.0% sorbitol and 0.2% locust bean gum (quantity 1500 mL, osmolarity 1148 mOsmol/L) with a solution combined with 2.0% sorbitol and 2.0% barium sulfate (quantity 1000 mL; osmolarity 194 mOsmol/L). The mean loop diameter after solution administration with higher osmolarity increased over time (up to 30 min). The side effect rate of both solutions was low, but the smaller amount was more acceptable for patients^[28]. Alexopoulou *et al.*^[29] used a total amount of 2.5 L of water solution containing 0.1 g/kg body weight of herbal fibres (psyllium) which was administered orally over a period of 4 h before MRI. This mixture has the property of retaining large amounts of water, up to 20 to 30 times its own volume, thus providing adequate distension. This agent also has biphasic properties, demonstrating low signal intensity in T1w images and high signal intensity in T2w images. MR enteroclysis-which the authors prefer-allows for dynamic filling of the small bowel. Thereby, relevant stenosis can

be detected dynamically. In this procedure, the patients will be provided with a tube inserted nasally, which will then be proceeded past the ligament of Treitz under fluoroscopic guidance, using a reduced pulse rate (rate 3 pulses/s; fluoroscopy time 1-3 min^[27]; authors' own experience: 4 s-1 min; Figure 2). The procedure of inserting and positioning of the catheter is the most challenging part of the entire procedure and can become a very unpleasant or almost intolerable maneuver for both the patient and the radiologist^[30]. Approximately 1000-1500 mL prewarmed methylcellulose will be administered manually during the MR scanning process over a time period of up to 15 min using 50 mL syringes. The filling can be automated by a pump which usually needs to be placed outside of the scanner room. The filling process will be followed by using a single-shot heavily T2w sequence with thick slabs (7-10 cm) in coronal orientation which are sequentially acquired during continuous filling of the bowel (Figure 3). This dynamic acquisition results in a film sequence of the filling process which can be helpful in the detection of atonic bowel segments, and hyperperistalsis, or in the delineation of stenosis and luminal diameter changes. If the patient suffers from nausea, the filling process needs to be stopped and may be continued after recovery. The total amount of methylcellulose tolerated is limited by patient compliance and by the amount of backflow of cellulose into the duodenum and the stomach. Filling is finished if the terminal ileum is widened and the caecum/ascending colon start to become distended. Necessity of additional placement of a transrectal catheter and filling of the colon depends on the region of suspected pathology^[31].

During the MR procedure the patients may be placed in the prone position, for a mild pressure to the anterior abdominal wall may result in better separation of the small-bowel loops. In case of MR enteroclysis this position will not be tolerated well. So, the authors suggest that the patient is placed in the standard supine position. In case of an unsuccessful separation of the loops the patient can be placed in the prone position at the end of the MR examination.

For MRI a dedicated multi-channel body array coil is used. The main technique involves MRI with ultra-fast T2w sequences supplemented by dynamic contrast enhanced T1w scans in combination with fat saturation covering abdomen and pelvis, from the lung bases to the perineum. True steady-state free precession images (TRUFPI) are obtained in the coronal plane for overview. T2w half-fourier acquisition single-shot turbo-spin-echo (HASTE) imaging is performed with long time of echo in axial and coronal orientation. T2w sequences are obtained with and without fat saturation. Fast low-angle shot T1w sequences (FLASH) are acquired in axial orientation. For dynamic multiphase contrast studies the authors use 3D sequences (T1-volume interpolated gradient-echo = VIBE) (Figure 4). Gadolinium-based agent at a dose of 0.1 mmol/kg will be administered intravenously. T1w VIBE sequences are acquired imme-

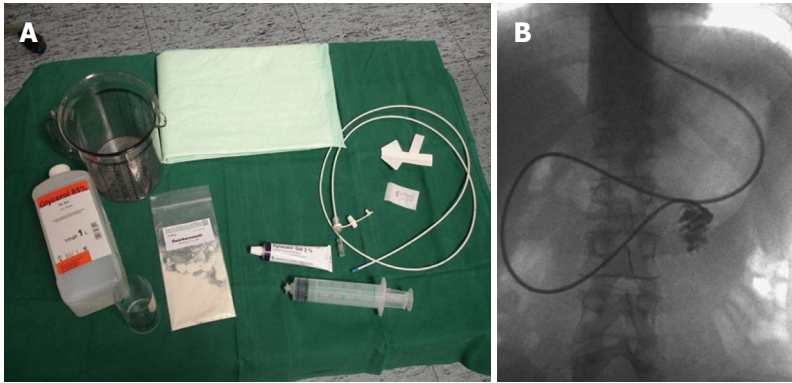


Figure 2 Magnetic resonance enteroclysis requires application of a nasojunal tube (A) and placement will be guided by pulsed fluoroscopy (B).

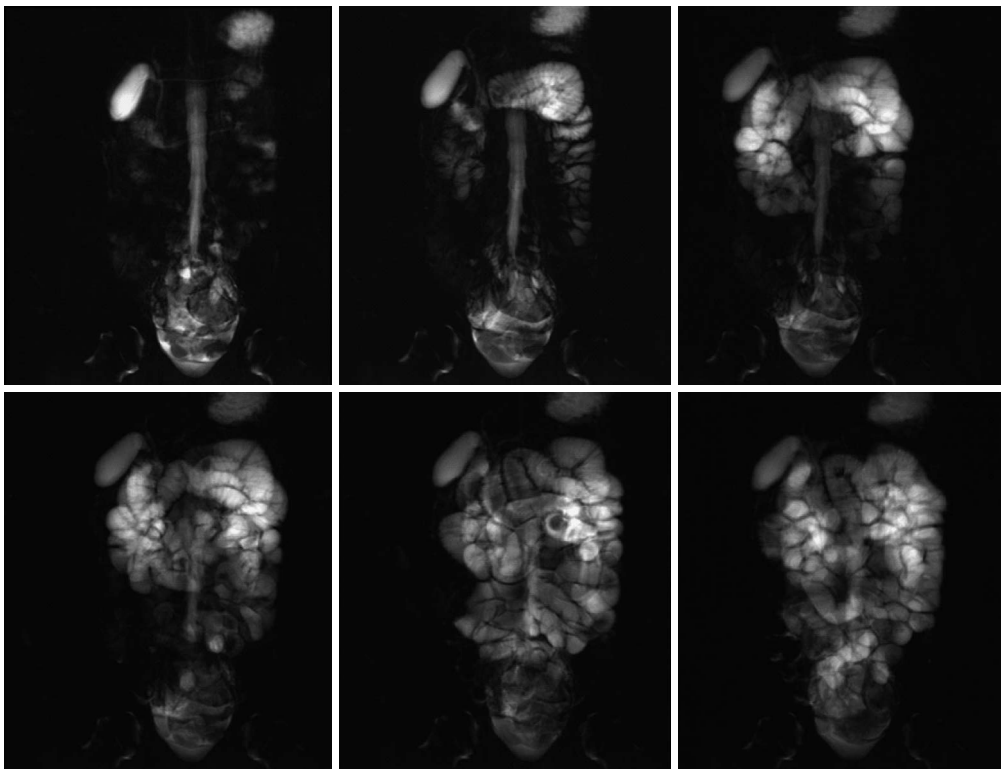


Figure 3 Enteral filling can be followed by using thick slab T2 weighted sequence (7-10 cm thickness).

diately after contrast application and repeated 30, 60, 90, and 180 s post injectionem (*p.i.*). Afterwards an axially oriented T1w FLASH sequence will be sampled in two stacks placed over epi- and mid-gastrium and over the pelvis. In case of suspected perineal/perirectal involvement further high-resolution sequences will be acquired in additional sagittal orientation, optionally with fat suppression (Table 2).

DISEASE EVALUATION USING MRI

MR imaging can detect a number of intestinal and extra-intestinal findings which should be included in the report (Table 3). First of all the intestinal distension and caliber changes have to be described. Afterwards intestinal and mesentery pathologies have to be mentioned.

Transmural abnormalities, wall thickening, loss of layering, cobblestoning, ulceration, pseudo polyps, mural abscess, pseudo sacculation appearance, stenosis, strictures, prestenotic dilation can be described without contrast application by assessing high resolution T2w sequences.

Thickening and pronounced enhancement of the intestinal wall

Bowel wall thickness of more than 3 mm is considered pathologic^[3]. Swelling of the wall is a result of interstitial edema. Increased signal in the bowel wall in edema sensitive T2w images (*e.g.*, sequence-short tau inversion recovery, STIR) may be suggestive for active disease (Figure 5). Hyperemia results in more or less increased signal intensity on T1w sequences after Gadolinium application, depending on disease activity (Figure 6). The degree of

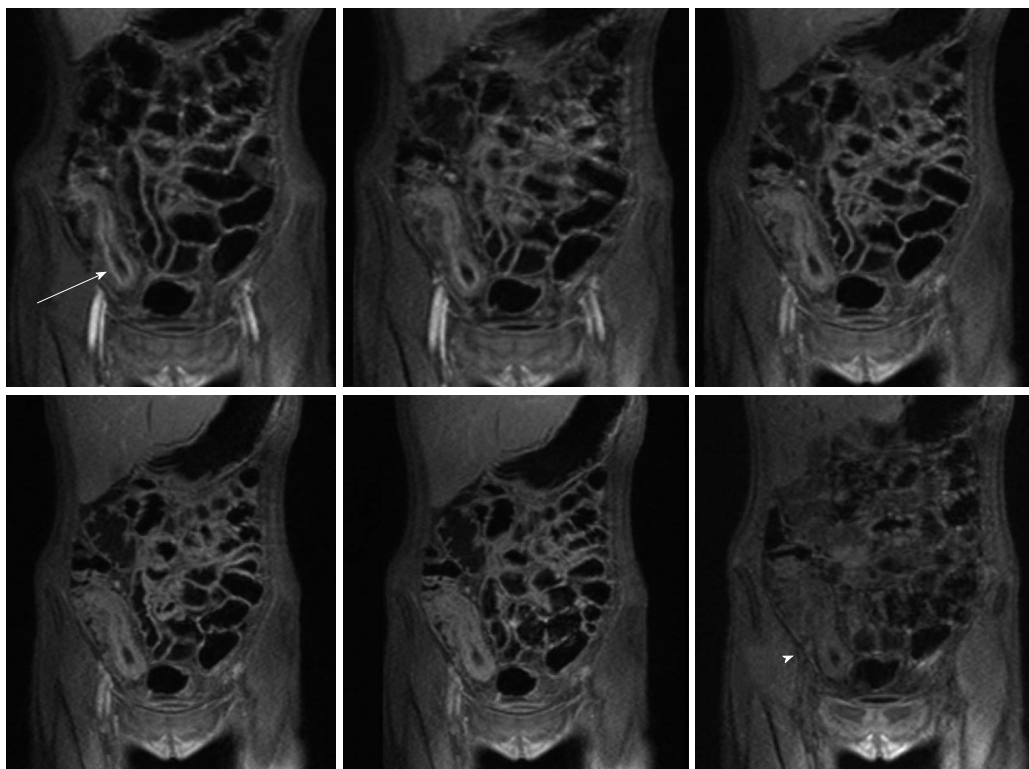


Figure 4 Dynamic contrast enhanced T1-volume interpolated gradient-echo sequence demonstrating different phases of signal increase in the various layers of the bowel wall [at first mucosa (arrow), followed by the serosa, the muscularis, and finally the submucosa (arrowhead)].

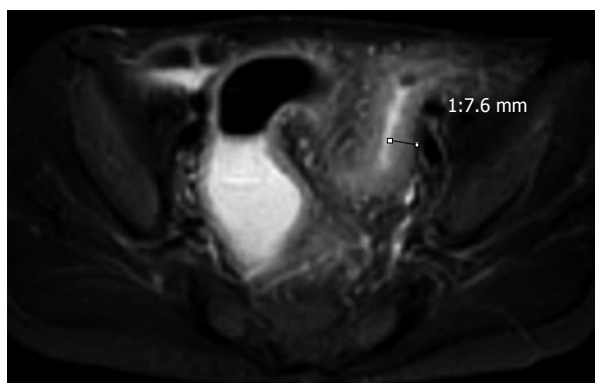


Figure 5 Eleven year old boy with Crohn's disease. T2 weighted sequence with fat saturation (Tirm/Stir) demonstrates thickening of the bowel wall and hyperintense signal corresponding to edema in acute inflammation. Narrowing of the lumen.

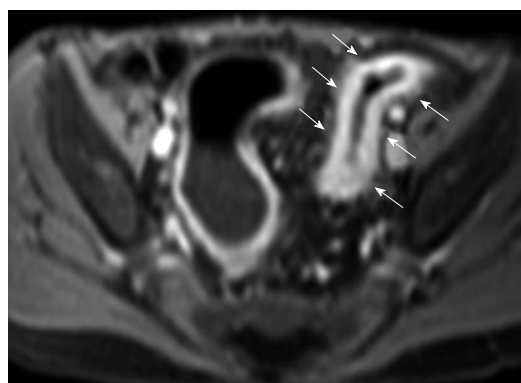


Figure 6 Eleven year old boy with Crohn's disease. T1 weighted contrast enhanced sequence shows strong transmurial enhancement (arrows).

bowel enhancement following contrast application (0.1 mmol/kg body weight Gd DTPA *iv*) by evaluation the increase in signal intensity (SI) can help to differentiate active from chronic disease^[29]. For quantification, Alexopoulos defined the percentage contrast enhancement as $\%CE = [(SI \text{ bowel postcontrast} - SI \text{ bowel precontrast}) / SI \text{ bowel precontrast}] \times 100$. A layered enhancement pattern on T1w scans can be highly specific^[29]. The use of contrast-enhanced MRI (CE-MRI) may be useful in diagnosing IBD, as well as in the differentiation between CD and UC^[32]. A study in children by Laghi *et al*^[33] correlated the semiquantitative score findings of MRI with endoscopic, histological, and CD activity index findings. MR

showed a sensitivity of 84% and a specificity of 100%. Other studies showed no correlation between percental contrast enhancement of the bowel wall and the CD activity index in children^[29]. New techniques like diffusion weighted imaging (DWI) are helpful adjuncts. DWI can be performed as transverse free-breathing echo planar imaging (EPI) sequence with diffusion-sensitizing gradients applied sequentially along the three orthogonal directions^[34,35]. Using a diffusion gradient of three different b values (0, 50, 800 s/mm²) the sensitivity and specificity compared to surgery and/or conventional enteroclysis was 86% and 97% in adults suffering from CD^[34]. Neubauer *et al*^[36] could prove DWI in combination with high-resolution T2w-HASTE is equal (if not superior) to CE-

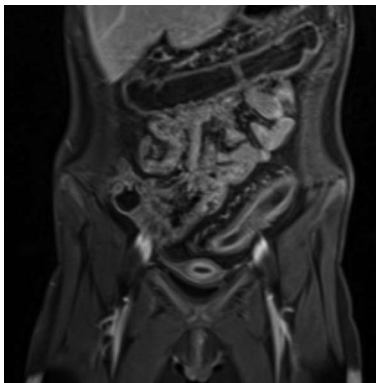
Table 2 Sequence parameters used for magnetic resonance enterography (magnetic resonance avanto, magnetic resonance symphony)

Sequence	Orientation	Slice thickness (mm)	Time of repetition (ms)	Time of echo (ms)	Time of inversion (ms)	Flip angle (°)
TRUF1	Coronal	6	3.8	1.9		80
T2	Coronal	70-100	3000	985		180
TRUF1	Transverse	4	4.3	2.15		80
HASTE	Transverse	4-7	1000	87		150
VIBE	Coronal	2	3.18	1.1		9
FLASH	Transverse	4-5.5	233	1.91		70
TIRM	Transverse	8	5050	91	140	180
DWI	Transverse	5	5500	139		0

TRUE: True steady-state free precession images; HASTE: Half-fourier acquisition single-shot turbo-spin-echo; FLASH: Fast low-angle shot T1w sequences; VIBE: Volume interpolated gradient-echo; DWI: Diffusion weighted imaging.

Table 3 Magnetic resonance imaging features in inflammatory bowel disease in children differentiation active and fibrostenotic phase

Imaging features	Active inflammation	Fibrostenotic disease
Mural thickening	Moderate	Mild
Mural enhancement	Avid	Mild
Stratified enhancement	Yes	Variable
Mural edema	Yes	Mild/absent
Mesenteric adenopathy	Yes	No
Fibrofatty proliferation	Variable	Yes
Abscess, empyema, fistula	Complicated disease	Variable

**Figure 7** Stratified enhancement in the sigmoid in a female suffering from ulcerative colitis.

MRI for detecting inflammatory lesions in children with CD. Based on these two sequences imaging without the need of contrast media seems to be sufficient for diagnosis, reducing scanning time to less than 10 min. Using a high b value of 800 s/mm^2 for DWI the background signal arising from non-inflamed tissue and from body fluids can be largely suppressed, so that inflamed bowel segments are more easily detected. The same authors observed a reduction of the apparent diffusion coefficient (ADC) in the inflamed bowel segments based by an altered diffusivity of extracellular water in inflamed bowel wall tissue. This could also be shown by another study in children with terminal ileitis using a b value of 500 s/mm^2 ^[37]. In this study there was a significant correlation between the bowel wall minimum ADC and established

MRI markers of disease activity (bowel wall thickening, striated pattern of arterial enhancement, degree of arterial enhancement, degree of delayed enhancement, amount of mesenteric inflammatory changes, and presence of a stricture).

Mural stratification

The inflammatory process in CD results in a complete loss of the intestinal layering which can be detected by high-resolution MRI. The transmural aspect of CD results in homogeneous high signal intensity of the entire bowel wall without normal stratification after administration of Gd. Exclusive involvement of the mucosa is a typical sign of UC. The result is a stratified enhancement the so-called target or double halo appearance (Figure 7). So, in the examination report the two patterns of enhancement can be described as “homogeneous” or “stratified”^[22]. The stratified enhancement pattern is typical for long-standing CD with fibrosis or in patients following intensive treatment.

Cobblestones and ulcers

Cobblestone formation is typically found in UC patients and less often in CD. The development of aphthous lesions along the mucosa result in deep linear or stellate lesions. Deep ulcers can be found in CD patients.

Strictures and stenosis

Whenever there is a procedural adequate distension of the bowel a reduction of the diameter (normally around 2.5 cm) is suspected a stricture. Chronic IBD-affected bowel segments may result in strictures. The presence of a pre-stenotic dilation may help in the diagnosis. Long-standing fibrotic strictures with a thick hypointense wall can be diagnosed on heavily T2w sequences. Notice that there is no significant contrast enhancement. Inflamed bowel segments tend to show a reduction of the size of the intraluminal diameter. The strictures can be demonstrated more sensitively using dynamic filling of the bowel with MR enteroclysis technique compared to hydro-MRI technique (Figure 8). In the case of lymph node bulking the bowel diameter can also be reduced. Description of localization and length of the involved bowel segment is necessary for planning surgery.

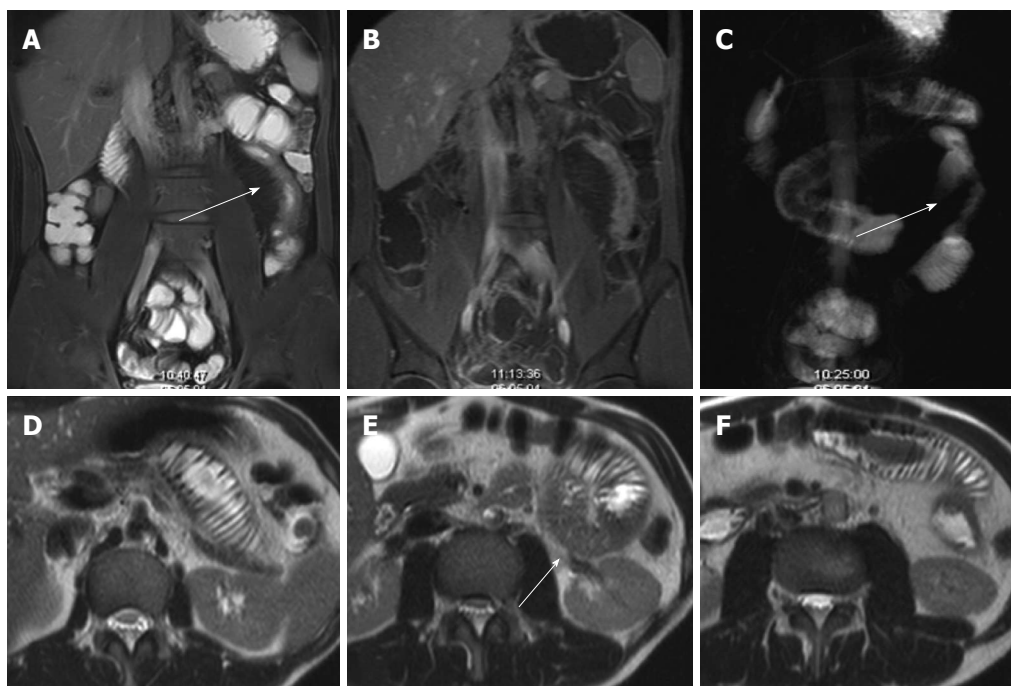


Figure 8 Stricture in inflammatory bowel diseases-chronic phase, no activity of inflammation. There is no edema with reduced signal intensity in T2 weighted sequences (A, D, arrow). The MR enteroclysmas does not show any widening of the involved jejuna bowel loop (C, arrow), also seen in axial T2w sequence (D-F). There is moderate enhancement (B).

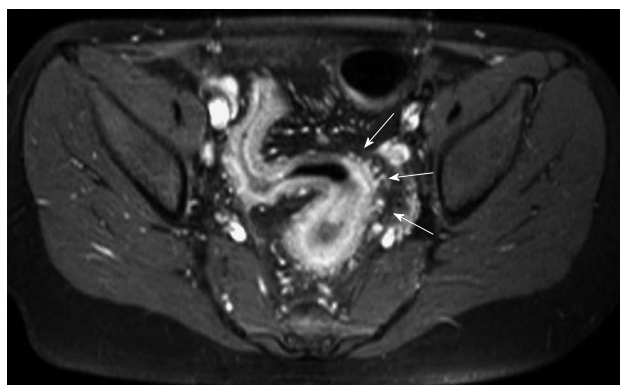


Figure 9 Twelve year old female with Crohn's disease. Comb sign. CE-T1 weighted sequence with fat saturation. Blood vessels within the mesentery (arrows).

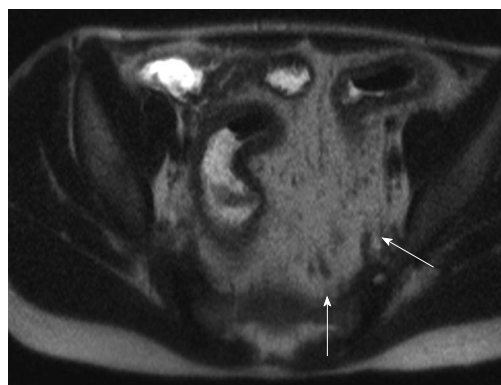


Figure 10 Eleven year old male with Crohn's disease. Creeping fat sign (arrows).

Extramural findings

The extramural signs of IBD such as free fluid, peritoneal fat stranding and enhancement, mesenteric edema, fibrofatty proliferation, the comb sign, mesenteric lymph nodes, abscesses, fistulas can be easily detected by MRI. Especially for the evaluation of perineal complications MRI is the gold standard nowadays. In a meta-analysis including 33 articles, Horsthuis *et al.*^[38] showed the highest sensitivity in MR enteroclysmas compared to hydro MRI, however, in children sensitivity was lower than in adults. One reason may be the lower amount of mesenteric fat which helps in separating the bowel loop segments for image interpretation. Motion artifacts, reduced compliance might be further possible reasons.

Comb sign

This is a sign of active inflammation (typical of CD) describing the vascular engorgement of the vasa recta on the mesenteric side of the bowel wall. The comb sign may be appreciated well on fat-saturated sequences as multiple tubular, sometimes tortuous vessels, aligned like the teeth of a comb (Figure 9).

Creeping fat sign

The chronic inflammation of the mesenteric fatty tissue induces a proliferation of the fat tissue itself together with a fibrotic component along the mesenteric border of inflamed bowel segments. This is the so called fibro-fatty proliferation which is also a typical CD sign. MR imaging can show a pseudo-mass which is surrounding the bowel

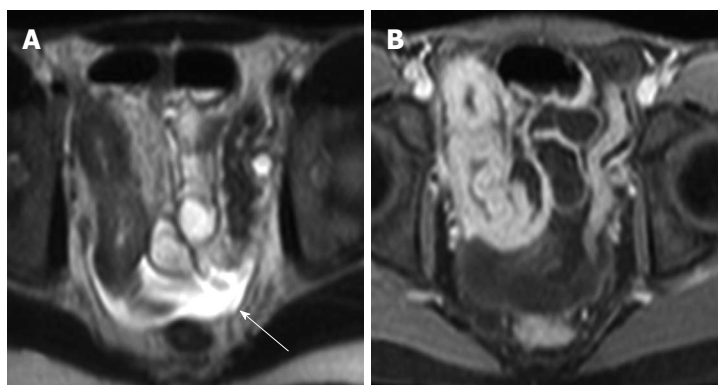


Figure 11 Thirteen years old girl with Crohn's disease. A: T2w image demonstrating strong hyperintense ascites. Arrow marks the free ascites; B: T1w sequence after contrast application. Fluid is not as easily detected as in T2w images. T1w: T1 weighted; T2w: T2 weighted.



Figure 12 Coronally oriented true steady-state free precession images shows enlarged mesenteric lymph nodes (arrows).

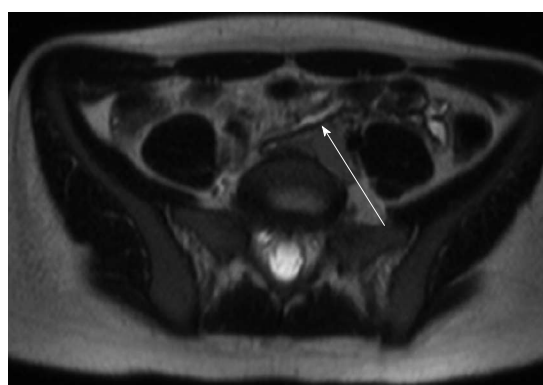


Figure 13 Seventeen years female with Crohn's disease. T2 weighted sequence showing enterocolic fistula (arrow).

loop with intermediate T2w signal intensity corresponding to fibrous or/and fatty components (Figure 10).

Free fluid

The distribution and amount should be described. Using the signal intensity on T1w and T2w sequences different entities (blood, pus, ascites) can be suspected (Figure 11). Diffusion weighted imaging is helpful in distinguishing serous fluid and empyema/pus.

Mesenteric lymph nodes

Mesenteric lymphadenopathy is well-depicted using TRUFI or T2w TSE sequences (Figure 12). In case there are multiple and round lymph nodes larger than 10 mm in diameter lymphoma needs to be excluded. As first choice follow-up imaging using ultrasound is recommended in these patients.

Fistula, abscess, phlegmon

Fistulas can be categorized as enteroenteric, enterocolic, enterovesical, enterocutaneous, or complex perianal (Figure 13). Fistulas are typically found in sites where two inflamed bowel segments are in close proximity to each other or in regions with high-grade bowel stenosis^[39]. Typically, the fistulous tract shows strong enhancement which can be differentiated using fat-suppressed T1w

high-resolution sequences. The direct visualization of the fistulous tract is not always possible, but indirect signs can be recognized. So, around the sinus tract inhomogeneity and enhancement of the mesenteric fat can be demonstrated. Other extraintestinal findings may be a psoas muscle abscess, and sacroiliitis (Figure 14). In these cases MRI of the pelvis will be recommended.

LIMITATIONS AND DEVELOPMENTS

The greatest limitation of MR enterography especially in children is the dependence on a compliant patient. It should be considered, that the patient is strained by fluid placement, application of contrast agents and motility influencing agents, and breath-holding maneuvers. Artifacts may be the result of bowel, breathing, and patient moving. In order to reduce these motion artifacts, two techniques can be applied: parallel imaging, navigator techniques, and motion correction^[40]. The use of higher field strength results in a greater signal-to-noise-ratio and has the potential of reducing scan time and increasing the spatial resolution^[5,28]. However, there is a concurrent increase in artifacts, especially chemical shift artifacts and susceptibility artifacts in abdominal imaging using gradient-echo imaging like TRUFI, CE-T1 VIBE, and FLASH. The T2w HASTE is more robust^[41]. Con-

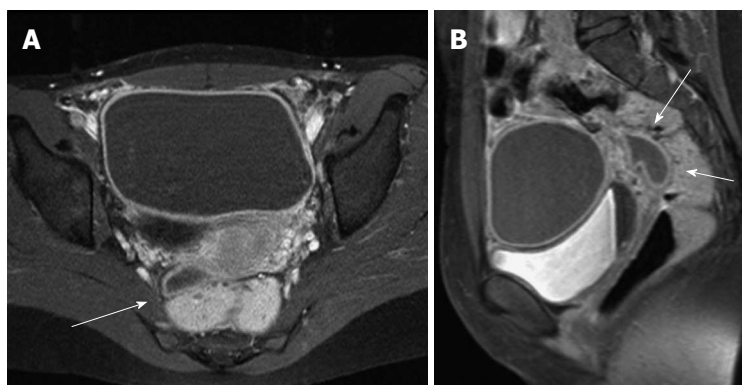


Figure 14 Female with Crohn's disease. Sagittal T1 weighted sequence allows to describe presacral abscess (arrow). Fat saturation was applied. A: Axial image; B: Sagittal image (arrows marking abscess; late contrast phase. Notice sedimentation of different components in the bladder).

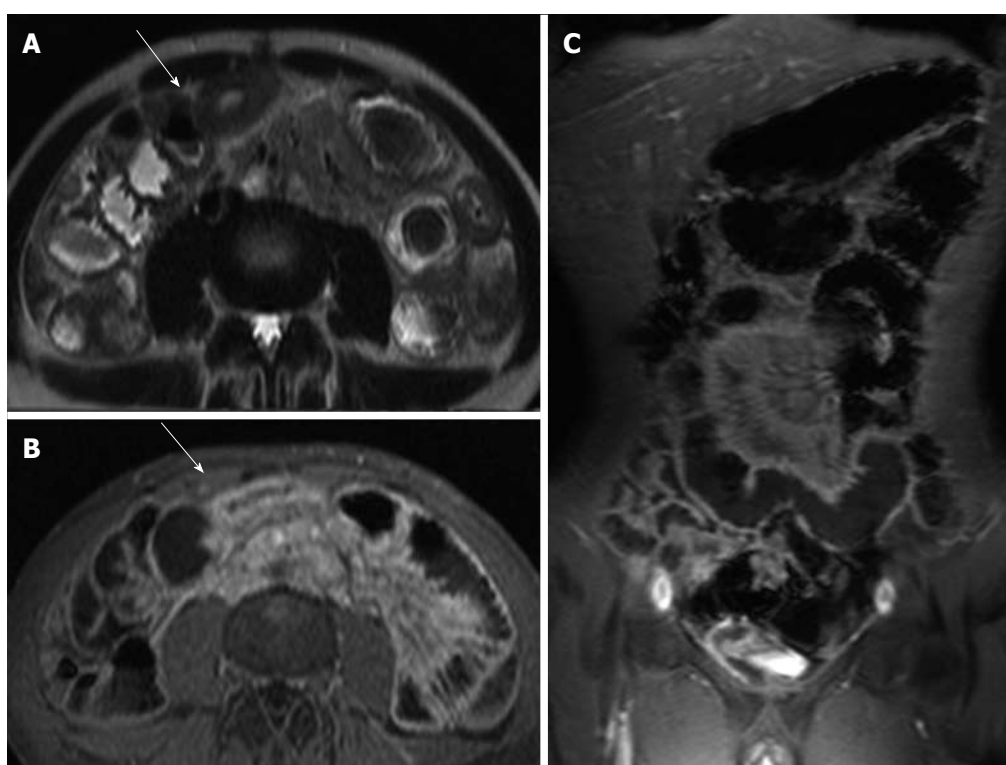


Figure 15 Magnetic resonance enterography allows an overview about involved bowel segments. In this case there was an isolated involvement of the jejunum. A: Axial T2w sequence showing thickened bowel wall without edema; B, C: Axial and coronal fat saturated contrast-enhanced T1w sequence demonstrating transmurals enhancement in Crohn's disease. T1w: T1 weighted; T2w: T2 weighted.

sequently, the feasibility of high-field MRI in children suffering from IBD should be studied in the future. The MR study is time consuming and staff-intensive. So, the use of a modified examination consisting of Diffusion weighted imaging with high b values from 600 to 1300 s/mm^2 and high resolution T2w sequences (isovoxel 3D with multiplanar reformation in three orthogonal directions) has to be evaluated in the future as a standard examination protocol resulting in a scanning time of not more than 10 min. This protocol can be used for routine controls. But, in the case of newly diagnosed IBD and in cases of relapse the examination has to be more extensive.

CONCLUSION

There are still sites that prefer fluoroscopic contrast studies and multislice CT in the diagnosis of IBD, also in children. But, the development of the last decade promises the increasing value of MR imaging in the evaluation of the intestine. MR enterography has definitely advantages including the detection and assessment of disease activity of the entire gut and the ability to evaluate extraluminal disease (Figure 15) and is therefore becoming the standard assessment of the small bowel in many centers. The radiation free ultrasound performed with adequate technique and experience by the sonographer should

be used as initial imaging method. MR imaging should be performed at the initial diagnosis of CD and should be considered in any case of treatment changes, especially if surgery is planned.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Role of the gut microbiota in inflammatory bowel disease pathogenesis: What have we learnt in the past 10 years?

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Abstract

Our understanding of the microbial involvement in inflammatory bowel disease (IBD) pathogenesis has increased exponentially over the past decade. The development of newer molecular tools for the global assessment of the gut microbiome and the identification of nucleotide-binding oligomerization domain-containing protein 2 in 2001 and other susceptibility genes for Crohn's disease in particular has led to better understanding of the aetiopathogenesis of IBD. The microbial studies have elaborated the normal composition of the gut microbiome and its perturbations in the setting of IBD. This altered microbiome or "dysbiosis" is a key player in the protracted course of inflammation in IBD. Numerous genome-wide association studies have identified further genes involved in gastrointestinal innate immunity (including polymorphisms in genes involved in autophagy: *ATG16L1* and *IGRM*), which have helped elucidate the relationship of the local innate immunity with the adjacent luminal bacteria. These developments have also spurred the search for specific pathogens which may have a role in the metamorpho-

sis of the gut microbiome from a symbiotic entity to a putative pathogenic one. Here we review advances in our understanding of microbial involvement in IBD pathogenesis over the past 10 years and offer insight into how this will shape our therapeutic management of the disease in the coming years.

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Gut microbiota; Innate immune response; Probiotics; Prebiotics; Faecal transplant

Core tip: In the last decade there have been enormous strides in our understanding of the role of gut microbiota in the aetiopathogenesis of inflammatory bowel disease (IBD). Newer molecular and genetic diagnostic tools have elucidated distinct changes in the gut microbiota in IBD patients and clarified the deficiencies of innate immunity. A link between environmental factors like diet, host immunity and the gut microbiota has been established. This review aims to enumerate these diverse strands of converging research in the last decade to outline the exciting prospects of possible personalized therapeutic interventions for patients with IBD in the coming years.

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INTRODUCTION

Inflammatory bowel disease (IBD) comprises two distinct

conditions, ulcerative colitis (UC) and Crohn's disease (CD) that are characterized by chronic relapsing inflammation of the gut in genetically susceptible individuals exposed to defined environmental risk factors^[1,2]. IBD was historically considered to be a "Western" disease but in the last decade there has been a definite increase in its incidence and prevalence suggesting that it is progressively emerging as a global epidemic^[3]. In the high prevalence regions the incidence of IBD has continued to rise in the past decade^[4,5].

There has been a parallel rise in our understanding of the critical role of the gut microbiota in the aetiopathogenesis of IBD. This is aptly exemplified by entering the key words, "microbiota" or "microflora" and "inflammatory bowel disease" into the PubMed database. On restricting the search to the last 10 years, over 800 articles published on this subject can be retrieved as opposed to 100 articles in the decade preceding it. This radical explosion of interest has been primarily due to the advent of culture-independent techniques like next generation sequencing and metagenomics which has enabled the global assessment of the gut microbiota much more accurately and in a vastly more sophisticated manner^[6,7]. The largest and perhaps the most ambitious initiative that has emerged in the last decade is the NIH sponsored Human Microbiome Project with a total budget of \$115 million to study the changes of the human microbiome in health and disease^[8]. It has recently led to the publication of 5177 microbial taxonomic profiles from a population of 242 healthy adults sampled at 15 or 18 body sites up to three times, with over 3.5 terabases of metagenomic sequence so far, which will serve as a comprehensive framework for future research in this field^[9].

This expansion of knowledge in the last decade has also shifted the search from external environmental triggers to a trigger within the complex luminal microbiome or the so called "in-vironment" that we harvest within ourselves^[10-12]. Prior to these radical developments research had focussed on unearthing a pathogen amidst the vast plethora of microbes in the gut lumen, which could be held responsible for initiating the inflammatory cascade that is typical of IBD^[13]. This endeavour was akin to searching for the veritable "needle in the haystack". The findings in the last decade has turned this whole concept on its head by revealing that the gut microbiome as a whole is altered in IBD, suggesting that perhaps the entire "haystack" is faulty. This concept of an altered gut microbiome or dysbiosis is possibly the most significant development in the field of IBD research in the past decade.

The other major shift in our knowledge of the aetiopathogenesis of inflammatory bowel disease has been from the host perspective. The dogma that CD and UC are typical autoimmune disorders was based on the characteristic histological appearance of these conditions and the response to immune-modulator drugs but the veil has lifted from this deep-embedded misconception^[14,15]. Over the past decade, genome wide association studies and newer genetic technologies have elucidated distinct genet-

ic defects in IBD patients. This has particular relevance with respect to host-microbial interaction at the luminal surface in the gut. A similar analysis on the PubMed database with the search items "genetics" and "inflammatory bowel disease" leads to a staggering yield of more than 5600 publications in the last decade as opposed to 2000 articles in the decade prior. It must be said that the avenue of research in this field was first opened up in 2001 when the first association of the nucleotide-binding oligomerization domain-containing protein 2 (NOD-2) gene mutation and susceptibility to Crohn's disease was documented^[16,17]. This has resulted in a drastic paradigm shift wherein IBD is no longer considered an autoimmune disease but may be an immunodeficient condition instead^[15]. This putative genetic susceptibility leads to a complex interaction between the diverse gut microbiome and the local innate immune system and forms the current basis for the aetiopathogenesis of IBD (Figure 1).

DYSBIOSIS

The normal gut microbiome comprises 100 trillion diverse microbes, mostly bacteria, encompassing over 1100 prevalent species, with at least 160 species in each individual^[18]. An exhaustive analysis of normal global gut bacterial communities suggests the possible existence of distinct enterotypes (*Bacteroides*, *Prevotella* or *Ruminococcus*) which are predominantly driven by dietary intake but independent of age or BMI^[19,20]. Further analysis suggests that the *Bacteroides* enterotype is associated with a "western" protein rich diet as opposed to the *Prevotella* enterotype which was associated with a carbohydrate rich diet^[21]. It remains to be seen whether this western enterotype turns out to be a distinct risk factor for developing IBD.

Dysbiosis or a definitive change of the normal gut microbiome with a breakdown of host-microbial mutualism is probably the defining event in the development of IBD. The shift from predominant "symbiont" microbes to potential harmful "pathobiont" microbes has now been well documented^[22]. Some of these changes in the gut microbiome have been detected in the common subset of IBD patients but some have been clearly delineated either in CD or in UC patients. The most well defined change that has been noted in patients with IBD is the reduced abundance of the phyla *Firmicutes*^[23-25]. Amongst the *Firmicutes*, the reduced presence of *Faecalibacterium prausnitzii* has been well documented in patients with CD as opposed to controls^[23,26-30]. This has been countered in a paediatric cohort of patients with CD where there were increased levels of *Faecalibacterium prausnitzii* suggesting a more dynamic role for this bacterium with a putative protective effect at the point of onset of IBD^[31]. In addition, there was a definite decrease in diversity of *Firmicutes*, with fewer of its constituent species detected in patients with IBD^[23,32,33]. Unlike *Firmicutes*, there have been reports of increased number of bacteria from the phylum *Bacteroidetes* in patients with IBD^[34-36]. Paradoxically, there

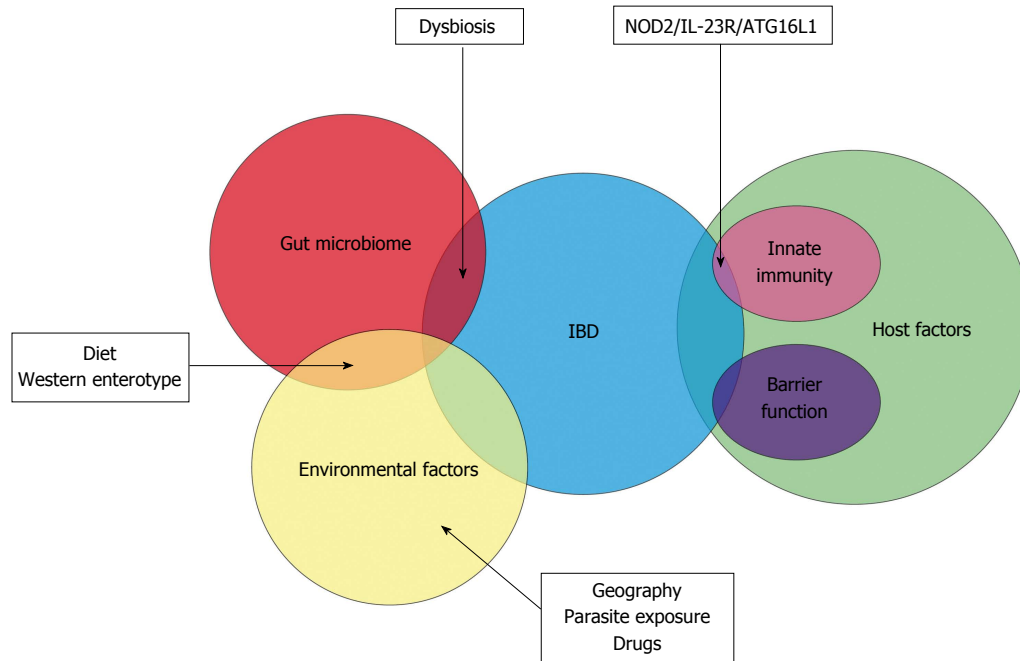


Figure 1 The Venn diagram depicts the overlapping role of the gut microbiome, host and environmental factors in the aetiopathogenesis of inflammatory bowel disease. Dysbiotic changes in the gut microbiome may be influenced by diet and other environmental factors and predispose to inflammatory bowel disease (IBD). A small proportion of IBD patients have demonstrable genetic susceptibility factors. NOD2: Nucleotide-binding oligomerization domain-containing protein 2; ATG16L1: Autophagy related protein 16-like 1; IL-23R: Interleukin 23 receptor.

have been some studies which have shown reduction in these bacterial species as well^[23]. There is a suggestion that there may be spatial reorganization of the *Bacteroides* species in patients with IBD, with *Bacteroides fragilis* being responsible for a greater proportion of the biofilm mass in patients with IBD compared to controls, suggesting increased adherence^[37]. Bacteria belonging to these two phyla make up for 90% of the phylogenetic categories in the normal microbiome and it is interesting to see the disparate ways in which they are altered in IBD.

Most of the known pathogenic bacteria in humans belong to the phylum *Proteobacteria*, which have been increasingly found to have a key role in IBD^[38]. Microbial diversity analysis has shown a shift towards an increase in bacterial species belonging to this phylum, suggesting an aggressor role in the initiation of chronic inflammation in patients with IBD^[39-42]. More specifically, increased concentrations of *Escherichia coli* including pathogenic variants have been documented in ileal CD^[28,43].

This interesting shift within the gut microbiome with a decrease in obligate anaerobes of the phylum *Firmicutes* and an increase in facultative anaerobes of *Proteobacteria* has given rise to a putative “oxygen” hypothesis wherein disruption in anaerobiosis points to a role for oxygen in intestinal dysbiosis^[44]. Similar functional disruptions associated with changes of the gut microbiome in patients with IBD may have more long reaching effects. Metagenomic analysis has revealed that the altered microbiome in IBD has 25% fewer genes and metaproteomic studies have shown a depletion of proteins and functional pathways^[18,45]. The ileal CD patients were found to have alterations in bacterial carbohydrate metabolism, bacterial-host

interactions, as well as human host-secreted enzymes^[45]. Elucidation of the functional impact of the changes seen as a result of dysbiosis will help design remedial measures that will help in the treatment of IBD patients.

The immediate question which follows is how the host responds to dysbiosis. Host genetics factors, specifically those pertaining to the innate immunity arm, is expected to play a role in the aetiopathogenesis of IBD. The “chicken and the egg” question is what comes first. Are the changes in the gut microbiome a result of an aberrant immune response in a genetically susceptible individual or does the abnormality in the gut microbiota lead to an aberrant immune response in such an individual? Twin studies have shown that disease phenotype rather than host genotype plays a greater role in determining changes in gut microbiota^[46]. However, studying the microbiota in subsets of patients with and without NOD2 and autophagy related protein 16-like 1 (ATG16L1) risk alleles showed that the affected genotypes were significantly associated with microbial compositional change but disease phenotype played a role as well^[47]. The confounding factor is that these two alleles are associated with ileal CD and not colonic CD. It makes it difficult to attribute these genetic defects as a cause of dysbiosis but highlights the intricate role of innate immunity in IBD.

INNATE IMMUNITY AND IBD

The gastrointestinal microbiota is a major source of immune stimulation. The colonic epithelium lies in close proximity to a high density of diverse microbes leading to a continuous network of communication between

host cells and microbes. This continual communication is essential for the maintenance of normal homeostasis though contribution to processes including supply of nutrients, xenobiotic metabolism and protection from pathogenic microorganisms, can have deleterious effects and contribute to intestinal inflammation^[48,49]. In patients with IBD this delicate balance is disturbed as a result of host immune defects in microbial recognition or handling/clearance strategies^[50]. Pattern recognition receptors (PRRs) are essential in distinguishing “friend from foe” in this very complex interaction and hold the key to understanding how genetic factors lead to an abnormal immune environment wherein normal commensal organisms can lead to pathological chronic inflammation. Ten years ago toll-like receptors (TLRs) and NOD2 were known to be involved in IBD pathogenesis although our understanding of their location, function and involvement was still very rudimentary. Evidence from IBD genetic studies had demonstrated that several innate immune genes had functionally relevant polymorphisms. Of those studied NOD2 genetic variants confer the greatest risk.

A decade ago the novel association between the recently characterised TLR4 Asp299Gly was described for both CD and UC^[51]. This finding supported previous evidence of PRR genetic influence in IBD susceptibility which had shown that polymorphisms in NOD2 (Arg-702Trp, Gly908Arg, and leu1007fsinsC) and the CD14-159C/T promoter polymorphism were associated with CD^[16,17]. Since then, additional polymorphisms in TLRs have been identified including TLR1 R80T and TLR2 R753G which have been associated with pancolitis in UC patients^[52]. The TLR9-1237T/C promoter polymorphism (TLR9-1237), which is associated with increased nuclear factor kappa B (NF- κ B) binding affinity, has also been associated with CD^[53,54].

The normal colonic epithelium constitutively expresses a variety of PRRs although expression levels are generally low with many receptors located basolaterally thus preventing interaction with luminal antigens^[55]. Nevertheless, intestinal epithelial cells are responsive to TLR ligands and recognise/respond to commensal bacteria secreting antimicrobial proteins and cytokines which facilitate intercellular interactions^[48]. Primary human intestinal epithelial cells express constitutive TLR3 and TLR5 and low levels of TLR2 and TLR4^[56]. TLR2, 4 and 5 are expressed on the cell surface and recognise extracellular microbes. TLR3 detects viral particles and is located intracellularly on early endosomal vesicles. The critical role of TLR4 as a first line of defence against potential bacterial pathogens is now beyond doubt. Impairment of TLR4 function permits bacterial invasion and persistence, and leads to the characteristic inflammation of IBD. The importance of TLR5 in intestinal homeostasis has also been effectively demonstrated using microbiota transfer from knockout mice^[57,58].

Distinct changes in TLR expression have been documented in IBD. TLR4 is found to be up regulated in

both UC and CD, whereas the levels of TLR2 and TLR5 remain unchanged^[56,59]. Altered TLR2 and TLR4 expression has been documented in the intestinal macrophages compared to peripheral monocytes, and a higher percentage of intestinal dendritic cells (DCs) have been shown to express TLR2 and TLR4 in IBD compared to control subjects^[59]. Intestinal macrophage signalling through PRRs has also been shown to be affected by increased expression of suppressor of cytokine signalling 1 and sterile and Armadillo motif-containing protein^[60,61]. Dysregulation of β -catenin and phosphatidylinositol-3-kinase pathways are also involved with alterations in these pathways involved in colitis susceptibility^[62,63]. In IBD patients increased cytokine production is seen by lamina propria DCs and macrophages, consistent with dysregulated tolerance^[64-66]. These changes can explain some of the abnormal response to the resident gut microbiota. However, it is difficult to elucidate whether the change in TLR expression initiates disease or is an epiphenomenon resulting from pro-inflammatory cytokine release. In many cases absence of epithelial cell-derived antimicrobial pathways increases susceptibility to intestinal inflammation, with IBD patients expressing lower levels of α -defensin compared to healthy individuals^[67,68].

Animal models had played a significant role in driving forward our understanding of IBD pathogenesis, especially murine colitis models. TLR4 and MyD88 knockout mice have been shown to demonstrate distinctly less pathology following chemical induction of colitis with dextran-sodium sulphate although bacterial translocation to mesenteric lymph nodes was more commonly detected^[69]. Impairment of TLR4 and TLR5 function has been shown to facilitate bacterial invasion and persistence (TLR4) and impact on intestinal homeostasis; development of metabolic syndrome (TLR5)^[57,58].

Alterations in NOD2 function due to genetic polymorphisms have demonstrated an inability to respond to bacterial muramyl dipeptide (MDP) leading to ineffective downstream signalling of NF- κ B^[70]. NOD2, is expressed on several different cell types including myeloid-derived, epithelial and endothelial cells. As with impairment of TLR function, NOD2 deficiency increases translocation of enteric bacteria to the lamina propria, with alterations in cytokine expression following exposure of peripheral blood mononuclear cells to MDP also reported potentially explaining the alterations in cytokine profiles typically seen in CD^[71,72]. Interestingly, NOD2 has more recently been shown to respond to viruses^[73]. With increasing interest in non-bacterial microbes in IBD pathogenesis, namely viruses and fungi, this may prove to be an increasing area of consideration. A decrease in the protective, anti-inflammatory Th-2 cytokine IL-10 has been documented in NOD2 mutants further adding to our understanding of the functional abnormalities characteristic of CD^[74].

Counter-intuitively, NOD2 can also contribute to down-regulation of inflammatory responses with chronic stimulation of NOD2 acting to tolerise cells against bac-

terial stimulation and ultimately down regulating other PRRs^[75-77]. Hence, in CD patients with dysfunctional NOD2 this restraint is removed and the inflammatory response from other PRRs increases.

NOD2 is also implicated in mechanisms of microbial killing. Autophagy, an important mechanism of microbial cell clearance, is regulated through PRRs^[78]. NOD2 interacts with ATG16L1^[78,79]. Therefore dysregulation of NOD2 impacts not only on microbial recognition but also handling. Genetic variants in ATG16L1 and also a second autophagy gene, immunity-related GTPase family M have been associated with CD^[80,81].

ROLE OF INDIVIDUAL PATHOGENS IN IBD

The rapid development of molecular techniques has also kindled hopes in the search for specific pathogenic agents initiating the inflammatory process of IBD. The pathophysiology of IBD does suggest that either primary or secondary pathogens play an important role in the cycle of inflammation. Many organisms have been proposed but those deemed to have been of the most interest over the last ten years are discussed below (Table 1).

Mycobacterium avium subspecies paratuberculosis

Mycobacterial infection has been postulated in the aetiology of Crohn's disease since its first description in 1913. The association stems from the observed similarity between Crohn's disease and the bovine condition Johne's disease, a condition caused by *Mycobacterium avium paratuberculosis* (MAP) infection leading to granulomatous enterocolitis. There have been vast numbers of studies in this area but the role of MAP remains uncertain^[50,82,83].

MAP can be widely isolated from meat, dairy products and water, indicating sources of infection and supporting its role^[82,83]. However, a large study found a lack of epidemiological support for environmental exposure^[84]. Over the last decade research into the prevalence of MAP in IBD patients has been inconclusive. A large number of researchers have successfully shown a higher prevalence of MAP in Crohn's patients compared to controls but, it seems for each of these there has been an equivalent study yielding no association^[85-97].

In support of its role the ability of MAP to invade gut epithelial cells, inducing tissue damage and inflammation, has been shown^[98]. A dominant T-cell response to MAP has also been seen in CD patients and macrophages infected with viable MAP are associated with high production of tumour necrosis factor- α (TNF- α), a marker for CD^[95,99,100]. Using mouse models, MAP has been found to induce full-thickness necrotizing colitis after subcutaneous and transluminal injection^[101].

The discovered association between CD and the autophagy gene *ATG16L1* lends further credence to the theory as it is known that autophagy is essential for inhibition of mycobacterium tuberculosis in infected macrophages^[102,103]. Defective innate immune killing mecha-

nisms in patients with *NOD2* polymorphisms at first also seem to support the idea, and indeed it has been found that monocytes heterozygous for a *NOD2* polymorphism are more permissive to the growth of MAP^[104]. Beyond contemplation, however, evidence for this hypothesis is limited. MAP has been detected most commonly in colonic disease; this is in direct contrast with the prevalence of *NOD2* mutation in ileal disease^[105,106]. In fact a study directly looking at the relationship between NOD-2 and MAP serology found no association^[107]. Combining this with the response of CD to immunosuppressant and anti-TNF therapy, known to cause MAP proliferation and a lack of success of anti-mycobacterial therapy, the role of MAP is clearly still in doubt^[108].

Helicobacter

Helicobacters, as human gastrointestinal pathogens, have assumed great research interest since the discovery by Robin Warren and Barry Marshall of *Helicobacter pylori* as the infectious agent in gastric and duodenal ulceration. Also, similar to MAP, one of the main prompts of research into the role of *Helicobacter* in IBD has been their propensity to cause colitis in animal models like Cotton-top tamarin monkeys (*Saguinus oedipus*). Despite this, there has been a lack of success in the last decade establishing presence of *Helicobacter* in IBD patients. The findings of studies looking into the molecular evidence of *Helicobacter* presence are varied and studies aimed at culturing viable *Helicobacter* from IBD tissue have failed^[109-121]. Interestingly the only seemingly universally accepted action of *Helicobacter* in IBD is the apparent protective effect of *Helicobacter pylori* which has convincingly been found to be negatively correlated with IBD^[113,118-122]. This may conform to the "hygiene hypothesis" for the development of IBD^[123].

The evidence for an association is much stronger with enterohepatic *Helicobacter* species. Non-*pylori Helicobacter* organisms have been shown to induce colitis in a number of rodent models; *Helicobacter hepaticus* and *Helicobacter bilis* (*H. bilis*) most prominently but, also *Helicobacter troglontum*, *Helicobacter rodentium* and *Helicobacter typhlonius* with cytokine patterns which were very similar to that of human IBD^[124-128]. When studying the response to *H. bilis*, Jergens *et al*^[126] showed that there was an IgG mediated response to the microbiota prior to the development of colitis, suggesting the ability of *H. bilis* to induce the hosts immune response to commensal bacteria, leading to the observed immune-mediated intestinal inflammation of IBD. In human subjects, enterohepatic *Helicobacter* species prevalence was significantly higher in colonic biopsy samples from patients with UC group compared to control subjects^[118].

Campylobacter

Campylobacter is a relatively new and important player in IBD. Unlike the other IBD suspects, *Campylobacter* does not have a suitable animal disease model; instead interest stems from the recognition of *Campylobacter jejuni* (*C. je-*

**Table 1 Evidence for the role of specific major pathogens in the aetiopathogenesis of inflammatory bowel disease in the last decade
n (%)**

Year	Pathogen	Disease	Sample type	Detection rate			Reference
				CD	UC	Control	
2003	MAP	CD	Tissue	34/37 (92)		9/34 (26)	[85]
2003	MAP	CD and UC	Tissue	0/24 (0)	1/28 (4)	6/19 (32)	[94]
2003	<i>Helicobacter</i>	CD and UC	Tissue	0/9 (0)	0/11 (0)	0/10 (0)	[109]
2004	MAP	CD and UC	Blood	107/283 (37.8)	50/144 (34.7)	135/402 (33.6)	[92]
2004	MAP	CD and UC	Blood	13/28 (46)	4/9 (45)	3/15 (20)	[86]
2004	<i>H. pylori</i>	UC	Tissue	8/42 (19)		7/74 (9.5)	[110]
2004	<i>Helicobacter</i>	CD and UC	Tissue	1/25 (4)	5/33 (15.2)	0/29 (0)	[111]
2004	<i>Helicobacter</i>	CD and UC	Tissue	0/30 (0)	0/26 (0)	0/25 (0)	[112]
2004	EHH	CD and UC	Tissue	3/25 (12)	3/18 (17)	1/23 (4)	[113]
	<i>H. pullorum</i>			2/25 (8)	0/18 (0)	1/23 (4)	
	<i>H. fennelliae</i>			1/25 (4)	3/18 (17)	0/23 (0)	
	<i>H. pylori</i>			8/25 (32)	5/18 (28)	14/23 (61)	
2004	<i>Helicobacter</i>	CD, UC and IC	Tissue	0/11 (0)	1/20 (5)	0/37 (0)	[114]
2004	<i>E. coli</i>	CD and UC	Tissue	11/14 (79)	8/21 (38)	10/24 (42)	[156]
	AIEC			10/14 (71)	10/21 (48)	7/24 (29)	
2004	AIEC	CD	Tissue	7/63 (11.1)		1/16 (6.3)	[154]
2004	<i>E. coli</i>	CD	Tissue	12/15 (80)		1/10 (10)	[158]
2006	<i>E. coli</i>	CD and UC	Tissue	9/12 (75)	7/7 (100)	2/8 (25)	[159]
2007	AIEC	CD and UC	Tissue	8/13 (61.5)	11/19 (57.9)	4/15 (26.7)	[160]
2008	<i>Helicobacter</i>	CD	Faeces	17/29 (59)		1/11 (9)	[115]
	EHH			11/29 (38)		1/11 (9)	
	<i>H. pylori</i>			6/29 (21)		0/11 (0)	
	<i>H. trogonum</i>			4/29 (14)		1/11 (9)	
	<i>H. canis</i>			5/29 (17)		0/11 (0)	
	<i>H. bilis</i>			4/29 (14)		0/11 (0)	
	<i>H. cinaedi</i>			1/29 (3)		0/11 (0)	
2009	AIEC	CD	Tissue	14/27 (51.9)		4/24 (16.7)	[155]
2009	<i>Helicobacter</i>	CD	Tissue	32/73 (43.8)		43/92 (46.7)	[116]
	EHH			18/73 (24.7)		16/92 (17.4)	
	<i>H. pylori</i>			29/73 (39.7)		39/92 (42.4)	
	<i>H. pullorum</i>			8/73 (11)		6/92 (6.5)	
	<i>H. canndensis</i>			10/73 (13.7)		10/92 (10.9)	
2009	<i>Campylobacter</i>	CD	Tissue	27/33 (82)		12/52 (23)	[131]
	<i>C. concisus</i>			17/33 (51)		1/52 (2)	
	<i>C. showae</i>			3/33 (9)		0/52 (0)	
	<i>C. hominis</i>			2/33 (6)		2/52 (4)	
	<i>C. gracilis</i>			2/33 (6)		0/52 (0)	
	<i>C. rectus</i>			1/33 (3)		2/52 (4)	
	<i>C. jejuni</i>			1/33 (3)		3/52 (6)	
	<i>C. ureolyticus</i>			1/33 (3)		2/52 (4)	
2010	<i>Helicobacter</i>	CD	Tissue	32/77 (41.6)		23/102 (22.5)	[117]
	EHH			18/77 (23.4)		12/102 (11.8)	
	<i>H. pylori</i>			14/77 (18.2)		11/102 (10.8)	
	<i>H. bilis</i>			1/77 (1.3)		1/102 (1.0)	
	<i>H. canis</i>			2/77 (2.6)		0/102 (0.0)	
	<i>H. hepaticus</i>			2/77 (2.6)		2/102 (2.0)	
	<i>H. trogonum</i>			5/77 (6.5)		4/102 (3.9)	
2010	<i>Campylobacter</i>	CD	Faeces	39/54 (72)		10/33 (10)	[132]
	<i>C. concisus</i>			35/54 (65)		11/33 (33)	
2010	<i>C. concisus</i>	CD and UC	Saliva	13/13 (100)	5/5 (100)	57/59 (97)	[136]
2011	<i>Helicobacter</i>	UC	Tissue		32/77 (42)	11/59 (19)	[118]
	EHH				30/77 (39)	2/59 (3)	
	<i>H. pylori</i>				2/77 (3)	9/59 (15)	
2011	<i>C. concisus</i>	CD, UC and IC	Tissue	8/12 (66.7)	3/8 (37.5)	11/26 (42.3)	[133]
2011	<i>Campylobacter</i>	UC	Tissue		51/69 (73.9)	15/65 (23.1)	[135]
	<i>C. concisus</i>				23/69 (33.3)	7/65 (10.8)	
	<i>C. ureolyticus</i>				15/69 (21.7)	2/65 (10.8)	
	<i>C. hominis</i>				14/69 (20.3)	5/65 (7.7)	
	<i>C. curvus</i>				3/69 (4.3)	4/65 (6.2)	
	<i>C. showae</i>				4/69 (5.8)	0/65 (0)	
	<i>C. jejuni</i>				2/69 (2.9)	0/65 (0)	
	<i>C. gracilis</i>				1/69 (1.4)	0/65 (0)	

2011	<i>Campylobacter</i>	CD and UC	Tissue	12/15 (80)	11/13 (85)	18/33 (48)	[134]
	<i>C. concisus</i>			10/15 (67)	9/13 (69)	12/33 (36)	
	<i>C. showae</i>			1/15 (7)	2/13 (15)	2/33 (6)	
	<i>C. hominis</i>			1/15 (7)	1/13 (8)	3/33 (9)	
	<i>C. ureolyticus</i>			2/15 (13)	1/13 (8)	2/33 (6)	
	<i>C. gracilis</i>			1/15 (7)	1/13 (8)	0/33 (0)	
	<i>C. rectus</i>			0/15 (0)	1/13 (8)	0/33 (0)	
	<i>C. jejuni</i>			1/15 (7)	0/13 (0)	0/33 (0)	
2012	AIEC	CD and UC	Tissue	1/17 (5.9)	1/10 (10)	0/23 (0)	[166]
2013	<i>Helicobacter</i>	CD and UC	Tissue	4/29 (13.8)	1/13 (7.7)	5/42 (11.9)	[119]
	<i>H. brantiae</i>			1/59 (3.4)	0/13 (0)	0/42 (0)	
	<i>H. hepaticus</i>			1/59 (3.4)	0/13 (0)	0/42 (0)	
2013	<i>Campylobacter</i>	CD and UC	Tissue	22/29 (75.9)	9/13 (69)	32/42 (76.2)	[119]
	<i>C. concisus</i>			13/29 (44.8)	4/13 (30.8)	16/42 (38.1)	
	<i>C. curvus</i>			2/29 (6.9)	0/13 (0)	3/42 (7.1)	
	<i>C. gracilis</i>			1/29 (3.4)	0/13 (0)	2/42 (4.8)	
	<i>C. hominis</i>			9/29 (31.0)	5/13 (38.5)	14/42 (33.3)	
	<i>C. rectus</i>			0/29 (0)	0/13 (0)	4/42 (9.5)	
	<i>C. showae</i>			9/29 (31.0)	5/13 (38.5)	9/42 (21.4)	
	<i>C. ureolyticus</i>			0/29 (0)	0/13 (0)	2/42 (4.8)	

CD: Crohn's disease; UC: Ulcerative colitis; IC: Indeterminate colitis; IBD: Inflammatory bowel disease; MAP: *Mycobacterium avium* subspecies *paratuberculosis*; EHH: Enterohepatic *Helicobacter*; E. coli: *Escherichia coli*; AIEC: Adherent-invasive *E. coli*.

jejuni) as the leading cause of gastroenteritis worldwide^[129].

The role of *C. jejuni* in human disease has been long recognised and its prevalence in IBD investigated^[130]. The main advance in the last decade has been the recognition of the importance of non-*jejuni* *Campylobacter* as human pathogens. Zhang *et al*^[131] found a higher prevalence of *Campylobacter concisus* (*C. concisus*) DNA and IgG levels in newly diagnosed paediatric patients with Crohn's disease, even managing to culture *C. concisus* from a biopsy sample, indicating viability. Another study using faecal samples from newly diagnosed CD patients also found a significant association with *C. concisus*, 35 of 54 CD patients testing positive and only 11 of 33 healthy controls. This study also found that *C. hominis* was present in 13% of Crohn's samples, *Campylobacter ureolyticus* in 9%, *Campylobacter showae* (*C. showae*) in 4%, *Campylobacter gracilis* (*C. gracilis*) in 2% and *C. rectus* in 2%. Interestingly *C. gracilis*, *Campylobacter rectus* and *C. showae* were only detected in patient samples^[132]. Similar results have been obtained in a number of studies in adult patients^[119,133-136]. Mahendran *et al*^[134] also showed an increased prevalence in UC, a finding supported by Mukhopadhyaya *et al*^[135] who found *C. concisus* DNA in biopsy samples in 23/69 (33.3%) of UC patients compared to 7/65 (10.8%) of controls. This study also found *C. ureolyticus* to be in higher prevalence in UC patients. The most recent study found that although *Campylobacter* appear to be surprisingly common, with positive PCR in 33/44 IBD patients and 32/42 controls, there was no association with IBD^[119]. A dominant serological antibody response to *C. concisus* has been documented in IBD patients indicating the prevalence of infection^[137,138]. Specifically CD patients have been shown to recognise flagellin B, ATP synthase F₁ subunit and outer membrane protein 18 of *C. concisus*^[137].

The origins of *Campylobacter* have led to a few researchers looking into the risks of developing IBD after acute gastroenteritis. A long term study published in 2009 documents the risk of developing IBD after acute

infection with *Campylobacter* (*C. jejuni*) or *Salmonella*^[130]. The findings indicated a significant increased risk in the exposed group for subsequently developing IBD, which has been supported by similar studies^[139-141].

The pathogenesis of *C. jejuni* had been fairly well established prior to the last decade. *C. jejuni* has been used to induce colitis in rodent models and previous exposure correlated with disease severity^[142]. The ability of *C. jejuni* to attach and invade the gut epithelium is well documented^[143]. The newest discovery has been that *C. jejuni* can promote translocation of commensal luminal bacteria. This is a natural process thought to be essential for immunological tolerance and mucosal surveillance in the GI tract. Up regulation could affect the normal mucosal response to the intestinal microbiota leading to the chronic immune-mediated intestinal inflammation of IBD^[144].

A number of studies have demonstrated the ability of *C. concisus* to colonise and adhere to intestinal epithelial cells, causing cell damage and microvillus degradation^[146]. Man *et al*^[145] comprehensively described the method of *C. concisus* attachment and invasion. They showed *C. concisus* to attach to the intracellular junction, disrupting barrier function - increasing permeability by causing a loss of tight junction proteins and decreasing transepithelial electrical resistance and to invade by a process mediated by polar flagellum^[146]. Other non-*jejuni* *Campylobacters* have also been shown to be invasive and induce pro-inflammatory cytokines as well as producing a number of virulence factors such as haemolysins, cytolethal distending toxin and zonula occludens toxin^[129,146-150]. These mechanisms could have an important bearing when one considers a causative role for this group of pathogens in IBD.

Adherent and invasive *Escherichia coli*

A specific pathogenetic group of *Escherichia coli* (*E. coli*), adherent-invasive *E. coli* (AIEC) have recently been extensively implicated in human IBD and are currently one of the most exciting players in the pathogen story. This

group are characterised by their ability to adhere and invade epithelial cells using actin microfilaments and microtubule recruitment. AIEC strains have been shown to be the cause of granulomatous colitis in boxer dogs and to induce granulomas, similar to early epithelioid granulomas, *in vitro*^[151-153]. Similarly to the previously discussed bacteria, they have been documented to induce colitis in infected animals.

There is a growing body of evidence supporting the prevalence of AIEC in human disease. A number of studies initially showed a disproportionate increase in *Enterobacteria* as a whole^[36,47]. When looking at AIEC organisms specifically, Darfeuille-Michaud *et al.*^[154] found them to be more prevalent in ileal Crohn's lesion tissue (36.4%) than controls (6.2%). This study also found that AIEC seemed to be rarely found in colonic tissue with 3.7% detected from Crohn's patients and 1.9% from controls, and none in UC specimens. This suggests a specific association of AIEC with ileal Crohn's. The findings of this initial study have been backed up by many researchers obtaining similar results^[28,155-161]. Additionally, antibodies to the *E. coli* membrane protein C and the CD associated bacterial sequence I2 have been shown to not only be more prevalent in CD but also to be associated with more severe disease, with small bowel involvement, faster disease progression and increased need for surgical intervention^[157,162].

The mechanism by which AIEC might induce colitis has been fairly well established towards the end of the decade. AIEC have type one pili and flagella that can bind to host adhesion receptor carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6)^[163,164]. CEACAM6 has been shown to be more highly expressed in ileal CD tissue, to be increased after- γ or TNF- α stimulation and to be upregulated by AIEC itself^[163,164]. AIEC have also been shown to possess long polar fimbriae and so can cross the mucosal barrier to access lymphoid cells^[165]. They can then invade macrophages without inducing cell death, allowing them to replicate and continuously activate immune cells, triggering TNF- α release and granuloma formation, which are hallmarks of Crohn's disease. In fact the use of TNF- α antibodies has been shown to decrease the number of intramacrophagic bacteria, relating this to the success of anti-TNF therapy and further supporting the role of AIEC^[166].

Other putative bacterial pathogens

In the last decade, there has been renewed focus in studying the role of various other bacterial strains in the aetio-pathogenesis of IBD as well. The role of *Fusobacterium* was studied in mucosal biopsies of patients with IBD and was found to be significant compared to controls in a number of studies prior to this review period. More recently seropositivity to *Fusobacterium varium* (*F. varium*) infection was found to be higher in UC patients as opposed to controls with increased severity of disease in seropositive UC patients^[167]. Further study has revealed the ability of *F. varium* to adhere to and invade colonic

epithelial cells, increasing IL-8 and TNF- α secretion, providing a mechanism whereby *F. varium* infection may induce inflammation similar to that seen in IBD^[168].

A similar association has also been found with *Klebsiella* infection, with Anti-*Klebsiella* antibodies found more commonly in IBD patients than in controls with the bacteria being implicated in disease relapses^[169]. *Klebsiella pneumoniae* has recently been shown to increase the severity of colitis in mouse models, increasing COX-2, IL-1 β , IL-6 and TNF- α expression and reducing tight junction associated proteins^[170]. Colitis has been shown to be induced even in wild type mice highlighting the high pathogenic potential of this bacteria^[171].

The role of *Salmonella* infection has been postulated from numerous studies that have documented the risk of developing IBD after acute *Salmonella* gastroenteritis^[130,139-141]. When searching for a mechanism it has been found that a *Salmonella* virulence factor, the invasion-associated type Darfeuille-Michaud secretion system induces inflammation by activation of the NOD1/NOD2 signalling pathways^[172]. This ties *Salmonella* nicely to the growing body of research into the genetics of IBD, supporting the role of the pathogen.

The potential role of *Yersinia* was proposed at the beginning of the last decade based upon the observed parallel increase in IBD and refrigeration, "the cold chain hypothesis"^[173]. Similarly to *Salmonella* and *Campylobacter* there has been evidence that acute *Y. enterocolitica* infection increases the short and long term risk of developing IBD^[174]. Despite some later successes in isolation *Yersinia* from IBD patients there has yet to be a compelling body of evidence coupling the prevalence of *Yersinia* with established IBD^[175-177].

MODULATION OF THE GUT MICROBIOTA AS A TREATMENT OPTION IN IBD PATIENTS

The past decade has seen rapid and definitive strides in determining distinct changes in the gut microbiota in patients with IBD. The effects seem to be global, involving not only the physical composition of the principal components but also significantly altering their function. The role of individual pathogens in this complex milieu still needs to be elucidated. The proof of concept of "dysbiosis" as an important step towards developing IBD needs to be proven with therapeutic trials attempting to reverse the process.

Role of probiotics and prebiotics

Probiotics are beneficial microorganisms that, when ingested, may influence the gut microbiota composition, metabolic activity and immunomodulation to confer benefit to the host^[178]. They can alter microbial diversity through competitive inhibition of other microbes, increase mucosal barrier function through the production of short chain fatty acids (SCFA) and interact with

intestinal DC to stimulate an anti-inflammatory response^[179-182]. These probiotic strains must be of human origin, be non-pathogenic and have the intrinsic ability to survive the gastrointestinal transit in order to confer maximal benefit^[183]. The most common probiotics used in the treatment of IBD have been *Lactobacillus* sp, *Bifidobacterium* sp, *Saccharomyces bouladrii*, *E. coli* Nissle 1917 and the probiotic combination VSL#3^[184-191]. The strongest indication for the use of probiotics in IBD has been in the treatment of pouchitis in the post-operative setting in UC patients^[192]. *E. coli* Nissle 1917 and VSL#3 have been found to be effective in preventing relapse and inducing remission in this setting^[193-198]. The data is not very robust with respect to the role of probiotics maintaining remission in UC and a recent Cochrane Database System Review has not recommended its use^[197]. The current body of evidence does not show any demonstrable benefit in patients with CD^[198,199].

Prebiotics are non-digestible oligosaccharides that are selectively fermented in the colon into SCFAs and can alter microbial composition and activity and confer benefit to the host^[200]. Examples include inulin, fructooligosaccharide (FOS), galactooligosaccharides and lactulose^[201-206]. Prebiotics can selectively stimulate the growth of certain probiotics such as *Lactobacillus* and *Bifidobacterium*, decrease intraluminal pH and increase the production of SCFA, such as acetate and butyrate, which play an important role in epithelial and DC function^[207,208]. SCFAs have also been found to have an anti-inflammatory effect^[209]. In an open labelled trial FOS use decreased the disease activity index in patients with active CD and resulted in increased faecal *Bifidobacterium*, but this benefit was not demonstrated in a subsequent randomized placebo controlled trial^[205,206]. Another prebiotic inulin showed some promise in a randomized controlled trial in patients with UC and was also found to decrease inflammation in patients with pouchitis^[202,203]. A couple of studies have also found a potential role of germinated barley foodstuff in maintaining remission in patients with active UC^[210,211]. Lastly, some benefit was also accrued with the use of Isphagula husk in patients with UC^[212]. The important studies and their brief outcomes are summarized in Table 2.

Antibiotics

A couple of recent meta-analyses on antibiotics in IBD found that the use of antibiotics improved clinical outcomes of patients with IBD^[213,214]. There is evidence that metronidazole and ciprofloxacin are useful in the treatment of CD and pouchitis^[215,216]. Support for the use of antibiotics as the primary treatment in UC is less convincing, however, there are some studies which suggest that rifaximin and ciprofloxacin could be useful as an adjunctive treatment for UC^[213]. The mechanisms through which antibiotics are thought to benefit patients with CD are through the inhibition of pathogenic bacteria or through reducing overall bacterial numbers. The main issues with antibiotic treatment include lack of understand-

ing of which bacteria may be involved in the initiation of inflammation, lack of specificity and the potential for antibiotic resistance. There have been several trials in the past studying the specific role of anti-mycobacterials in the treatment of CD and this has been summarized in a European consensus document which has deemed the futility of such treatment^[217].

Faecal transplantation

Faecal transplantation or faecal microbial therapy (FMT) as it is more commonly known is a technique in which stool is taken from a healthy surrogate and inserted into an unhealthy person, with curative intent^[218]. The origins of faecal transplantation as a method of treating enteric pathology can be traced back for more than two millennia, when it was used as a traditional Chinese medicine to treat diarrhoea^[219].

In recent times, FMT is perhaps best known for its potential role in treating *Clostridium difficile* (*C. difficile*) infectious diarrhoea^[220]. After donor-faeces infusion in a group of patients infected with *C. difficile*, there was an alteration in the gut microflora with an increased faecal bacterial diversity, similar to that in healthy donors, with an increase in *Bacteroidetes* species and *Clostridium* clusters and a decrease in *Proteobacteria* species. This therapeutic benefit by FMT as documented in this trial, would theoretically have a beneficial effect in patients with IBD as well. The use of this form of intervention is still restricted to few exploratory trials. Donor faecal enemas were given to a group of ten children over five days with moderate to severe UC and resulted in 78% clinical response after a week and 67% with sustained response after a month in the nine children who could tolerate the treatment^[221]. This was similarly documented in a subset of six adult UC patients who were treated over a period of 5 d. Complete reversal of symptoms was achieved in all patients by 4 mo, by which time all other UC medications had been ceased and at 1 to 13 years post FMT and without any UC medication, there was no clinical, colonoscopic, or histologic evidence of UC in any patient^[222]. However, a single infusion in six adult patients with severe UC did not have a similar beneficial effect and the faecal microbiota changed to the donor phenotype in only 50% of those treated, suggesting that as opposed to treatment of *C. difficile* a prolonged treatment is indicated in IBD^[223]. Although the data described above is certainly promising, there is clearly a need to move on from individual case reports and conduct more large scale randomised control trials before any benefit of FMT can be claimed with any certainty. Some concerns have also been raised regarding safety and side effects, with some IBD patients suffering mild side effects following FMT, and the obvious issues surrounding potential transmission of host infectious disease^[224]. The efficacies of different administration techniques and dosing regimens for FMT also need to be refined and investigated. Literature to date describes a range of methods including colonoscopy, duodenal or gastric tubes and self-administered

Table 2 Probiotics and prebiotics in inflammatory bowel disease

Active component	Study	Design	n	Duration	Intervention	Result	Reference
<i>Lactobacillus</i>	CD remission	RCT	98	6 mo	<i>Lactobacillus johnsonii</i> LA1 4×10^9 cfu/d	No difference	[184]
	IBD		40	1 mo	<i>Lactobacillus rhamnosus</i> GR-1 and <i>L. reuteri</i> RC-14 supplemented yogurt	Anti-inflammatory effects	[185]
<i>Bifidobacterium</i>	Active UC	RCT	20	12 wk	Bifido-fermented milk [<i>B. breve</i> , <i>B. bifidum</i> and <i>acidophilus</i>] (1×10^{10}) or placebo	Decreased clinical activity ($P < 0.05$) decreased endoscopic/histological scores ($P < 0.01$)	[186]
	Active UC	Open label	12	4 wk	BGS 4.5 g/d	Decrease in clinical activity index ($P < 0.01$) and endoscopic scores ($P < 0.05$)	[187]
	C57BL/6 mice	Experimental	16	3 d	<i>B. bifidum</i> S17	Decrease in microscopic inflammation and reduction in inflammatory cytokines	[188]
<i>E. coli</i> Nissle 1917	UC remission		327	12 mo	200 mg <i>E. coli</i> Nissle 1917 or 1500 mg mesalazine/d	<i>E. coli</i> Nissle 1917 was equivalent to mesalazine in maintaining remission	[189]
VSL#3	UC remission	Open label	34	6 wk	VSL#3, 3.6×10^{12} , bacteria/d	ITT analysis demonstrated remission in 18/34 and response in 8/34	[190]
	Active UC	RCT	29	12 mo	VSL#3 450-1800 billion bacteria/d	Remission was achieved in 13/14 VSL#3 and 4/15 placebo ($P < 0.001$) Relapses within 1 yr of followup occurred in 3/14 VSL#3 and 11/15 placebo Endoscopic and histological score were significantly lower in VSL#3 vs placebo ($P < 0.05$)	[191]
Inulin	Active UC	RCT	19	2 wk	3 g/d mesalazine and either 12 g/d oligofructose-enriched inulin or placebo	Dyspeptic symptoms scale decreased significantly and an early reduction of calprotectin was observed in oligofructose-enriched inulin group	[202]
	Pouchitis	RCT	20	3 wk	24 g/d inulin or placebo	Reduction in inflammation, increase butyrate conc and decreased inflammation associated factors	[203]
Inulin and FOS	HLA-B27 rat model IBD			12 wk	8 g/kg body weight inulin or FOS	FOS increased <i>Bifidobacterium</i> spp. FOS and inulin reduced <i>Clostridium</i> cluster XI and <i>C. difficile</i> toxin gene expression correlating with a reduction of chronic intestinal inflammation	[204]
FOS	Active CD	RCT	103	4 wk	15 g/d FOS or placebo	No clinical benefit, despite impacting on DC function	[205]
	Active CD	Open label	10	3 wk	15 g/d	Significant reduction in Harvey Bradshaw index ($P < 0.01$) significant increase in faecal bifidobacteria conc. ($P < 0.001$) and modifies DC function	[206]

CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; RCT: Randomized control trial; FOS: Fructooligosaccharides; *E. coli*: *Escherichia coli*.

enema yet to date there is no clear evidence to support one method over any other. There is no doubt that manipulation of the gut microbiota could have enormous therapeutic potential and FMT will play an important role in its future.

CONCLUSION

The understanding of the aetiopathogenesis of IBD has undergone radical shifts in the past decade with the advent of modern molecular techniques that can character-

ize the gut microbiome more accurately and host genomic analysis that can explore the vast genetic universe of IBD. At the heart of the inflammatory process in IBD is "dysbiosis" of the gut microbiome, which may be driven by host genetics and environmental factors like diet. The next decade will help unravel the intricacies of the host immune defences that determine this intriguing host-microbiome ecology. The relationship of the genotype of the host and the extent to which it determines the composition of the microbiome needs to be elucidated. It will open the doors to more "personalized" therapeutic

interventions, which would encompass the host genotype and serotype, the disease phenotype, the gene expression profiles of the immune cells and the microbiome composition to decide the best strategy for treating patients with IBD. This will usher in a paradigm shift in patient management with a move away from standard generic therapy to a scientific, tailored approach based on the needs of individual patients.

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Therapy with stem cells in inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) affects a part of the young population and has a strong impact upon quality of life. The underlying etiology is not known, and the existing treatments are not curative. Furthermore, a significant percentage of patients are refractory to therapy. In recent years there have been great advances in our knowledge of stem cells and their therapeutic applications. In this context, autologous hematopoietic stem cell transplantation (HSCT) has been used in application to severe refractory Crohn's disease (CD), with encouraging results. Allogenic HSCT would correct the genetic defects of the immune system, but is currently not accepted for the treatment of IBD because of its considerable risks. Mesenchymal stem cells (MSCs) have immune regulatory and regenerative properties, and low immunogenicity (both autologous and allogenic MSCs). Based on these properties, MSCs have been used *via* the systemic route in IBD with promising results, though it is still too soon to draw firm conclusions. Their local administration in perianal CD is the field where most progress has been made in recent years, with encouraging results. The next few years will be decisive for defining the role of such therapy in the

management of IBD.

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Key words: Mesenchymal stem cell; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Hematopoietic stem cell transplantation

Core tip: Treatments with mesenchymal and hematopoietic stem cells offer a potential that requires in-depth investigation. Existing studies are encouraging yet inconclusive. We are at a point of inflexion where these new therapies are seen to afford major curative potential. The coming years will be decisive. The information obtained from ongoing and future clinical trials may lead a revolution in inflammatory bowel disease management and its impact upon patients. Undoubtedly, as twenty-first century gastroenterologists, we must expand the scope of our specialty and seek multidisciplinary interaction for the benefit of our patients.

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main conditions found in inflammatory bowel disease (IBD). The incidence and prevalence of IBD have gradually increased in recent years. It is estimated that in Europe 1.4 million and one million people may have UC and CD, respectively^[1]. In the United States over 1.3 million people have IBD^[1]. Furthermore, in the last few decades there has been an increase in the disease in low-incidence zones as South Korea, China, India, Iran

Lebanon, Thailand, the French West Indies, North Africa and Japan^[2,3]. IBD poses an important health problem, since its worldwide incidence is increasing^[1], the condition affects young people, and persists for life - exerting a strong impact upon quality of life, in the professional setting, and in patients' personal relations^[4,5]. Furthermore, IBD is associated with considerable healthcare costs^[6,7].

Although the etiology of IBD remains unclear, there have been significant improvements in our knowledge of its physiopathology, allowing advances in treatment and a change in the current therapeutic objectives. Before the introduction of anti-tumor necrosis factor- α (anti-TNF α) drugs, the management of IBD was centered on symptoms control. Drug substances (mesalazine, corticosteroids, thiopurines, methotrexate and cyclosporine) were used to induce and maintain disease remission. Although endoscopic disappearance of the lesions was known to occur in CD patients subjected to thiopurine therapy^[8], the significance of mucosal healing was not known, and consequently did not constitute a therapeutic objective. The introduction in 1998 of anti-TNF α drugs for the treatment of CD improved the clinical outcomes of therapy, and mucosal healing was found to be associated with a good prognosis, fewer hospital admissions and surgical operations, and improved quality of life^[9]. Later studies of both CD and UC showed mucosal healing to be a therapeutic objective^[10-17]. In this context, current treatment focuses on achieving "deep remission" of IBD (symptoms control and endoscopic healing of the mucosal lesions)^[14,15,18,19]. The purpose of treatment is to control the symptoms and the underlying intestinal inflammatory process (which is responsible for the progressive intestinal damage), and to restore normal intestinal function. Accordingly, in CD we aim to avoid structural intestinal damage, reduce disability over the long term, and improve patient quality of life. In the case of UC, we aim to reduce the percentage of colectomies and the incidence of colorectal cancer, and improve patient quality of life. All these strategies are aimed at changing the natural course of the disease.

However, despite the efforts being made to optimize use of the existing drugs, the current situation is far from ideal. Approximately one-third of all CD patients fail to respond to anti-TNF α therapy (primary non-responders)^[9,11,20,21], and 10% of all CD patients do not tolerate or are primary non-responders to all the drugs used for treating the disease. Among the individuals who do respond to anti-TNF α treatment, one-third show transient loss of response and require either optimization or a switch to another biological agent (secondary non-responders)^[22]. Furthermore, despite the new therapeutic strategies, the number of surgeries in CD remains stable^[23]. Esophageal cancer (EC) is the second leading cause of intestinal transplantation in adults, as a consequence of the development of intestinal failure secondary to multiple surgical operations. In this context, disease recurrence in the graft is the norm^[24,25]. Perianal disease can be a serious problem that proves difficult to

control with the existing treatments, and refractory cases are subjected to aggressive surgery associated with a considerable psychological impact for the patient^[26,27]. In addition to the above, it must be mentioned that IBD is becoming more common in the pediatric population, and patients now report to the clinic as adults with a history of multiple intestinal resections and immune modulating treatments with or without biological agents. In this population group, gastroenterologists predict that there may be a loss of response over the long term, and the future of these patients thus appears uncertain. Lastly, in some patients who do not respond to medical treatment, surgery is unable to solve the problem due to the location and extent of the lesions.

The situation is different in the case of UC, since in severe disease flare-ups and in patients resistant to medical treatment, costly surgery in the form of colectomy is a therapeutic option.

All this leads us to seek new treatment options. In recent years, efforts have increasingly focused on the study and use of cell therapies with T lymphocytes^[28], tolerogenic dendritic cells^[29] and both hematopoietic and mesenchymal stem cells (MSCs). The present review examines the role of stem cells in the control of IBD. An analysis is made of the physiopathological principles of stem cell therapy, the results of the studies carried out to date, and the future perspectives in this field.

PHYSIOPATHOLOGY OF IBD

Advances in our knowledge of the pathogenesis of IBD are the basis for the development of new treatments.

At present, IBD is regarded as the result of an abnormal host immune response to intraluminal antigens occurring in a genetically predisposed individual, with the production of chronic inflammation of the gastrointestinal tract, accompanied by tissue destruction. IBD is the consequence of complex interaction among genetic^[30,31], environmental^[1] and microbial factors^[32], producing sustained inflammation at intestinal level, favored by alteration of the mucosal barrier and immune system defects^[33].

Under normal conditions, the intestine presents minimal physiological inflammation, despite exposure to a large number of intraluminal microbial and food allergens, and the presence of a significant number of lymphoid cells (80% of all lymphocytes are located in the intestine)^[33,34]. This situation is the result of complex mechanisms involving innate immunity^[35-39], represented by the epithelial mucosal barrier, innate cell immunity (leukocytes, monocytes, macrophages and dendritic cells), and innate humoral immunity (*e.g.*, lysozyme, complement and interferons). While this innate response is not specific, it is able to differentiate so-called pathogen-associated molecular patterns, and to activate different intracellular mechanisms that condition or orientate the adaptive response. On the other hand, dendritic cells serve as the link between innate and adaptive immunity, presenting antigens to naïve Peyer's patch lymphocytes and inducing

their differentiation^[40]. The Lymphocytes are the main effector cells of the adaptive immune response, and are activated at intestinal level to eliminate pathogenic antigens. Once the antigens have been eliminated, the lymphocytes are down-regulated, thereby maintaining intestinal homeostasis^[38]. Lymphocytes are memory cells, and when they are exposed again to an already identified antigen, the immune response is both faster and more potent than at first exposure. The physiological mechanisms whereby the intestine differentiates pathogenic and non-pathogenic antigens, and the pathways involved in the correct maintenance of intestinal homeostasis, are not clear. We do know that a balance between regulatory T cells (Treg) and effector T cells (Th1, Th2 and Th 17) is essential for intestinal homeostasis^[41,42].

It remains unclear whether the inflammatory process damages the mucosal barrier and thus allows penetration of the intraluminal antigens, thereby activating the inflammatory process, or whether mucosal barrier alteration is the primary event that in turn triggers the inflammatory response.

In recent years there have been major advances in the knowledge of the aspects that are believed to be involved in the development of IBD.

The genetic bases of IBD were recognized early in clinical practice in view of the increased incidence of the disease in homozygous twins, in first-degree relatives, and in certain ethnic groups^[43-46]. At present, genome-wide association scan studies (GWAS) have identified over 100 loci related to the development of IBD^[47]. A GWAS meta-analysis has revealed 77 genes associated to CD, the existence of shared genes between CD and UC^[30], and 47 loci related to UC^[31]. This suggests there are overlapping genes in both disease conditions which predispose to the development of IBD [genes with expression products that encode for the interleukin (IL)-23 pathway, transcription factors such as NK2, locus 3 (NKX2-3), SMAD3, STAT3, ZMIZ1 and c-REL], by encoding for proteins involved in the innate and adaptive immune responses^[48-50]. Likewise, there are genes implicated in the development of CD such as NOD2, and genes that regulate autophagia^[51,52], while other genes are specifically associated to UC, such as the genes located on chromosome 6p21 (related to the major histocompatibility complex), or to mucosal barrier integrity and defense^[47]. On the other hand, genetic factors have been shown to be associated to the phenotype of the disease, its pattern, evolutive course and response to drug treatment^[53-57]. Genetic disorders in IBD are multiple and complex, and unfortunately we are able to predict less than 25% of hereditary correlations in IBD^[58]. The exception is represented by IL-10 receptor alteration, which is associated to severe CD, and seen in children refractory to treatment and with a good response to allogeneic hematopoietic stem cell transplantation (HSCT)^[59].

Epidemiological studies have shown that environmental factors are essential for the development of IBD, and could account for the increase in the presence of the

disease in developing countries^[1]. These environmental factors could exert a direct influence upon changes in the intestinal microbiota, or on the appearance of new intraluminal antigens from foods or environmental toxic agents. Diet and antibiotics are the most important determining factors of bacterial diversity in the gastrointestinal tract, and their modification runs parallel to the socioeconomic development of countries^[60]. The microbiota plays a key role in the development of IBD among genetically susceptible hosts^[61]. In this regard, a recent publication addresses the possible infectious origin (produced by an unknown bacterium) of a case of colitis with histological data indicating chronicity and the presence of granulomas. The condition is considered idiopathic, and occurs in patients subjected to umbilical cord allogeneic hematopoietic cell transplantation^[62]. This opens a door to the future search for microbial agents in similar disease conditions characterized by an unknown etiology.

Lastly, intestinal homeostasis is lost in IBD as a result of defects in the intestinal epithelial barrier and/or imbalances between Treg and effector T cells (Th1, Th2, Th17) - giving rise to an inappropriate immune response to harmless intraluminal antigens (components of the diet) or intraluminal bacteria^[42,63].

It is currently accepted that the predominant inflammatory profile in CD involves a type Th1 and Th17 activated CD4⁺ lymphocyte response, with an increase in interferon- γ (IFN- γ), TNF α , IL-17 and IL-22^[64]. In UC, the cytokine profile is similar to that of the natural killer (NK) cell mediated Th2 response, producing IL-5 and IL-13. This latter interleukin is crucial for the development of UC, exerting cytotoxic action against the epithelial cells and positive feedback upon the NK cells, resulting in tissue damage^[64,65].

Improved knowledge of the pathways leading to inflammation, and of the implicated cytokines, cells and adhesion molecules, have allowed the identification of many therapeutic targets upon which to act. Until the causal agent can be identified, these are the advances which can help us in the search for new drug substances.

Stem cell therapy aims to modify the immune response of patients with IBD and repair the tissue damage caused.

STEM CELL THERAPY

Stem cells are characterized by asymmetrical division, giving rise to a cell with the same properties as the original cell (self-renovation), and another cell of multilineage potency that can differentiate in one way or another depending on environmental conditions.

There are different types of stem cells, depending on their origin and functions^[66].

Embryonic stem cells

These are pluripotent cells obtained from embryos, and which can produce all the tissues derived from the three embryonic layers both *in vitro* and *in vivo*.

Table 1 Two types of stem cell therapy are currently used for the treatment of inflammatory bowel disease

Type of therapy	Mechanisms of action
Hematopoietic stem cells	Autologous transplant: Elimination of reactive T lymphocytes (lymphoablation, new reconstitution of the immune system of the patient with more tolerogenic naïve lymphocytes). The genetic predisposition of the patient is not modified Allogenic transplant: Replacement of the immune system with the donor immune system, correcting patient genetic predisposition. Not accepted due to high morbidity-mortality
Mesenchymal stem cells (autologous or allogenic)	Systemic and local administration: Immune-modulating and trophic action

Multipotent adult stem cells

These cells are found in all body tissues, where they may have reparatory functions. Hematopoietic stems cells (HSCs) and MSCs belong to this category. Both types are found in bone marrow, though MSCs can be obtained from different tissues (bone marrow, adipose tissue, umbilical cord, placenta, *etc.*), do not produce hematopoietic cells, and can differentiate towards cells belonging to the mesenchymal lineage.

Induced pluripotent stem cells

These are defined as artificial pluripotent stem cells^[67] that can be generated from somatic cells following the introduction of reprogramming factors (OCT314, SOX2, KLF4, c-MYC, NANOG and LIN28). These cells acquire the same properties as pluripotent stem cells, and their phenotypic differentiation can be redirected according to the culture media used. These cells pose no ethical problems, since they are not of embryonic origin. As a result, they are currently under study for the development of organs and the repair of tissues carrying the genome of the sick patient^[68].

The use of embryonic stem cells and induced pluripotent stem cells has been associated to the development of teratomas following transplantation. This fact, and the present impossibility of eliminating undifferentiated cells produced as a result of division, poses a serious problem for clinical use^[69].

Two types of stem cell therapy are currently used for the treatment of IBD: hematopoietic stem cell therapy and MSCs therapy (Table 1). Most treatment evaluations are made in CD patients, since in UC patients surgery is an option in the case of resistance to therapy.

HEMATOPOIETIC STEM CELL THERAPY IN IBD

Hematopoietic stem cells are immature cells found in bone marrow, the bloodstream and the umbilical cord. They present glycoproteins as CD34⁺, CD 90⁺, CD38⁺, CD133 at surface level and can differentiate towards cells

pertaining to hematological cell lines^[70]. In clinical practice, HSCT was initially used to treat hematological malignancies. Subsequently, as a result of experimental studies in animals^[71,72] and the clinical improvement experienced by patients with immune-mediated diseases (IMDs) subjected to HSCT due to malignant conditions^[73,74], HSCT has become increasingly used in selected patients with IMDs refractory to conventional therapy^[75,76]. The treatments used at present in application to IBD aim to control the inflammatory process but do not act upon the origin of the disease. The therapeutic objective of autologous HSCT would be to restore the primary immune system of the patient (resetting of the immune system), after using chemotherapy to eliminate self-reactive T lymphocytes (lymphoablation) and memory cells, which would constitute the effectors of the immune dysregulation observed in CD^[77], thereby inducing antigen tolerance over the long term. Based on studies carried out in other IMDs^[78,79], the changes of the new immune system could be the result of reactivation of thymus gland activity (with the restoration of new polyclonal T cells) and the *de novo* induction of Treg derived from the thymus, which would be essential for restituting tolerance of harmless antigens^[80-82]. Genetic factors are crucial in the development of IBD, and are not modified by autologous HSCT. IBD might reappear after exposure to triggering antigens, and the body would probably respond in the same way. However, and in the worst of cases, the available therapies may be prescribed earlier in an attempt to change the natural course of the disease, or while waiting for the development of new treatments.

In any case, allogenic HSCT can correct the genetic defects of the patient by conforming a new immune system (that of the donor), associated to ablation of the immune cells of the recipient^[77]. This transplant strategy is currently not accepted as primary treatment for IMDs, due to its high associated mortality and complications rate.

In 1995, an international committee produced guidance with the criteria and protocols for performing HSCT in patients with severe IMDs, including IBD^[83]. The committee recommended autologous HSCT instead of allogenic transplantation, due to its lower risk and toxicity.

HSCT in IMDs is carried out following the protocol used in the treatment of hematological malignancies. In autologous HSCT, the hematopoietic stem cells are obtained from peripheral blood in 95% of all cases. To this end, a first mobilization step is carried out, stimulating the production and release of stem cells from the BM towards the peripheral blood compartment. The treatment scheme most widely used in CD is cyclophosphamide 1.5-2 g/m² and granulocyte colony stimulating factor (G-CSF) 10 µg/kg per day^[84-86]. Peripheral blood stem cells are then collected through apheresis followed by cryopreservation until HSCT is performed. Before re-infusing the hematopoietic stem cells, some work groups use local protocols to purify cell culture, selecting CD34⁺ cells or selectively eliminating lymphocytes by means

of monoclonal antibodies (anti-CD52, anti-CD3, anti-CD19 or anti-CD20)^[84]. Other groups do not eliminate lymphocytes^[85]. Finally, the conditioning stage (elimination of self-reactive T cells) is carried out, using cyclophosphamide and anti-thymocyte immunoglobulin^[86], and hematopoietic cell infusion is carried out at the end of this stage.

The conditioning regimens used vary according to the autoimmune disease and the treating center^[87]. Non-myeloablative conditioning regimens have been specially designed for IMDs. In the case of IBD, lymphoablation is performed without irreversible destruction of the hematopoietic cells of the BM, and marrow function could be recovered without the infusion of hematopoietic stem cells. Reinfusion is performed to shorten the duration of BM aplasia. The reasons for not using myeloablative regimens would be safety in first place, since lymphoablative protocols present fewer complications and a lower mortality rate. On the other hand, IBD recurrence after autologous HSCT is highly probable, since the immune system of the patient is regenerated. In turn, following a very aggressive initial phase, some IMDs undergo spontaneous remission for reasons that are unknown, and in these patients such aggressive therapy would be unnecessary^[81].

Autologous HSCT in EC

The first case reporting the efficacy of autologous HSCT in the control of CD was published in 1993, and corresponded to a patient with non-Hodgkin lymphoma^[88]. Subsequently, isolated cases of IBD improvement in patients receiving autologous HSCT for malignancies were published^[89-92]. In 2003, the Chicago group published the first series of CD patients subjected autologous HSCT as primary treatment for IBD^[93]. A later publication by this same group^[84] reported the results of autologous HSCT (phase I trial) in 12 patients with CD and EC activity index (CDAI) scores of 250-450, refractory to conventional treatment, including infliximab. Eleven patients presented disease remission, defined by CDAI < 150, with a mean follow-up of 18.5 mo (range 7-37 mo). In 2008, Cassinotti *et al*^[85] published the clinical results of autologous HSCT in four CD patients. Clinical and endoscopic remission was observed in three subjects after 16.5 mo of follow-up (phase I - II trial). Unlike the Chicago group, these authors do not perform CD34⁺ cell selection before hematopoietic stem cell infusion. Since then, some isolated cases as well as larger patient series have been published in which the primary objective of HSCT has been the control of refractory CD^[82,94-96]. In the series published by Hasselblatt *et al*^[96], 56% of patients achieved endoscopic healing of mucosal lesions after cell transplantation - these data being similar to those obtained in the phase III trials with anti-TNF α ^[9-11]. The study carried out by Oyama *et al*^[84] reported improvement of CD following the cell mobilization phase with low dose cyclophosphamide and G-CSF, and suggested that the improvement may have been due to the action of chemotherapy rather HSCT. However, Cassinotti reported worsening of CD in

three of the four patients in the period between stem cell harvesting and transplantation. The phase III Autologous Stem Cell Transplantation International Crohn's Disease (ASTIC) study, sponsored by the European Crohn's and Colitis Organisation (ECCO) and the European Group for Blood and Marrow Transplantation (EBMT), was designed to clarify this issue. The patients included in this study were randomized to two treatment arms: mobilization chemotherapy with G-CSF and autologous HSCT in 30 d *vs* mobilization chemotherapy with G-CSF and conditioning with autologous HSCT after 13 mo. The preliminary results have been presented in the ECCO of 2013, concluding that HSCT appears to be effective in CD patients, affording endoscopic improvement of the lesions. However, it involves a risk of adverse effects, and the end results referred to the trial objective are still awaiting analysis^[97].

It is difficult to know the precise results of autologous HSCT in CD, due to the few patients treated to date. In the series published by Burt *et al*^[94], which is the most numerous to date, all patients ($n = 24$) entered remission (CDAI < 150) after transplantation. Over subsequent follow-up, the percentage of patients in remission without the need for CD treatment was 91%, 63%, 57%, 39% and 19% after one, two, three, four and five years, respectively. Regardless of the medication taken, the percentage of patients annually in remission in the 5 years after transplantation was 70%-80%. Eighty percent of patients were in corticosteroid-free remission each year, in the 5 years after transplantation. Regarding the safety of the procedure, infectious complications during the first year were the most significant problem, though mortality was zero (one patient died in an accident).

The overall safety of HSCT in the treatment of autoimmune diseases can be assessed from the two large recently published registries: that of the EBMT, and the registry of the British Society of Blood and Marrow Transplantation (BSBMT). The results of the observational study of the EBMT, documenting the course of 900 patients subjected to autologous HSCT between 1996-2007, showed the 5-year survival rate to be 85%, with a patient disease-free rate of 43% - though there were important variations depending on disease type. In the multivariate analysis, mortality was found to be related to the experience of the center ($P < 0.003$) and type of autoimmune disease ($P < 0.03$). An age of less than 35 years ($P < 0.004$), transplantation after the year 2000 ($P < 0.0015$), and type of autoimmune disease ($P < 0.0007$) were associated to a disease-free patient course^[87].

In 2012, Snowden *et al*^[86] published the results on HSCT in patients with IMDs documented in the BSBMT registry between 1997-2009 (70 transplants in 69 patients: 55 autologous cell transplants and 15 allogenic transplants). The survival rate in the case of autologous transplants was 85% in the first year and 78% after 5 years. The corresponding figures for allogenic transplantation were 87% and 65%, respectively. The disease-free rate for autologous transplants was 51% in the first year and

33% after 5 years. The corresponding percentages for allogeneic transplantation were 80% and 65%, respectively. There were no differences in mortality in the two groups. The leading cause of death was infection, while age was strongly correlated to survival (95% survival after 5 years among patients between 18-39 years of age).

Autologous HSCT may be a valid option in patients with treatment-refractory CD. This therapy must be provided at specialized centers where correct patient screening is performed, and in which complications referred to both transplantation and autoimmune disease are known and can be treated. Further studies are needed to determine the precise role of this procedure in CD treatment.

Allogeneic HSCT in IBD

No descriptions were found in the literature of allogeneic HSCT as primary treatment for CD until 2009, when this technique was first used to treat a patient with mutation of the *IL10RA* and *IL10B* genes (encoding for proteins ILR10R1 and ILR102, which form part of the IL-10 receptor). This mutation was identified in homozygosis in three children from the same family that developed proctitis early (at 3 mo of age), with severe perianal disease refractory to treatment. Following allogeneic HSCT, sustained CD remission was observed^[59].

This treatment is not currently accepted as primary therapy for IBD and, with the exception of the aforementioned case, the results found in the literature correspond to patients with neoplastic disease associated to IBD, in which the course of the latter changed after HSCT. The most numerous allogeneic HSCT series to date is that published by Ditschkowski *et al*^[98]. In this series, 11 patients (7 with CD and 4 with UC) underwent allogeneic HSCT due to hematological malignancies. All patients were subjected to myeloablative treatment and total body irradiation (except in two cases). One patient died of sepsis, while the rest were found to be in complete neoplastic disease remission after 34 mo of follow-up, with no clinical relapse of IBD.

Lopez-Cubero *et al*^[99] previously described 6 CD patients diagnosed with leukemia and subjected to allogeneic HSCT. Five of them had active CD before transplantation, and two had sclerosing cholangitis. All of them received cyclophosphamide in the total body irradiation scheme. One patient died of sepsis three months after transplantation. Four of the remaining 5 patients remained symptom-free during a period of 54-183 mo after transplantation. The patients with a clinical diagnosis of primary sclerosing cholangitis showed improvement in alkaline phosphatase levels.

Similar findings have been described in a patient with CD and acute myeloid leukemia subjected to allogeneic HSCT^[100].

This procedure is not currently recommended in IBD, except in very specific cases such as mutation of the *IL-10* gene commented above. Its efficacy and risks have been described in the above section.

MSC THERAPY IN IBD

MSCs, also known as stromal cells, are adult pluripotent cells initially described by Friedenstein *et al*^[101], who isolated them from bone marrow. They are able to adhere to plastic surfaces, and can differentiate *in vitro* into chondrocytes, osteoblasts and adipocytes. The International Society for Cellular Therapy established the minimum criteria that must be met in order to classify a stem cell as an MSC^[102]: (1) adherent to plastic under standard culture conditions; (2) express CD105, CD73 and CD90; (3) lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and human leukocyte antigen (HLA)-DR; and (4) differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*.

These cells have been identified in bone marrow, adipose tissue, connective tissue, umbilical cord and placenta, and it is known that they are found in “niches” in different tissues - including the intestine^[103,104]. In most clinical studies and in human therapy, MSCs are obtained from bone marrow, adipose tissue and umbilical cord. Adult bone marrow has been widely used to obtain MSCs for clinical purposes, though their presence in adult marrow is low (0.001%-0.01%), extraction is difficult, and the procedure also poses risks for the donor. Furthermore, the differentiation capacity of these cells decreases with donor age, and expansion must be performed in culture media prior to administration of the cells. As a result, alternative MSC sources have been explored^[105]. A larger number of MSCs can be obtained through liposuction; *in vivo* expansion is not required; cells can be administered directly; and their properties are similar to those of MSCs obtained from bone marrow^[106-108]. A recent study published by Melief *et al*^[109] shows that both types of cell have similar immune modulating functions, though MSCs of adipose tissue origin present a different cytokine secretion profile, and their immune modulating effects are more potent than those of MSCs obtained from bone marrow.

The use of MSCs is based on their potential capacity to repair damaged tissues and inhibit inflammation and fibrosis^[110,111]. In addition, the administration of MSCs of both autologous and allogeneic origin requires no conditioning phase, since these cells are not immunogenic: they express low levels of class I HLA antigens at surface level and do not express type II HLA antigens or T cell coactivators^[112]. However, in immunocompetent mice it has been seen that the half-life of these cells is notoriously shorter following second exposure. This suggests they do not evade the immune system entirely, and can be rejected^[113-115]. These properties have defined MSCs as potentially useful for the treatment of autoimmune diseases and in those processes in which tissue repair is needed^[112].

The action mechanism is based on cell contact and paracrine action involving the release of soluble factors (Figure 1).

As regards the immune regulating properties of the

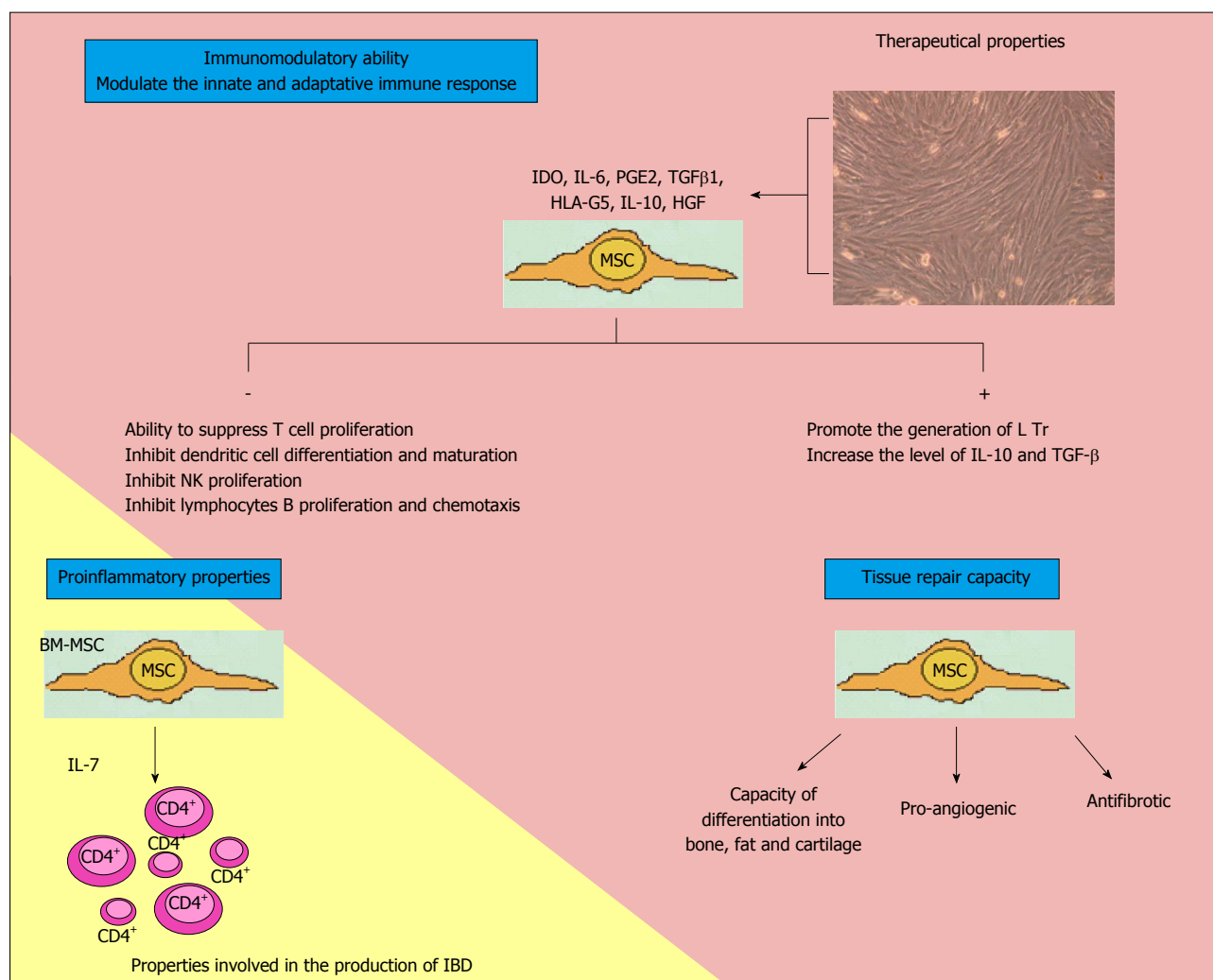


Figure 1 Characteristics of multipotent mesenchymal cells. MSCs: Multipotent mesenchymal cells; IDO: Indoleamine 2,3 dioxygenase; IL: Interleukin; PGE2: Prostaglandin E2; TGFβ1: Transforming growth factor beta-1; HLA-G5: Human Leucocyte Antigen G5; HGF: Human growth factor; NK: Natural killer; Tregs: Regulatory T cells; BM-MSC: Bone-marrow-mesenchymal stem cell; CD4⁺: Activated lymphocytes CD4; IBD: Inflammatory bowel disease.

cells, they are capable of regulating both innate and adaptive immunity^[116]. Cell culture studies have shown that MSCs are able to suppress T cell proliferation^[113]. However, MSCs do not possess intrinsic immunosuppressive capacity; instead, such capacity is acquired when cells are stimulated by proinflammatory cytokines such as INFγ, TNF-α and interleukin-1β^[117]. Duijvestein *et al*^[118] have shown MSC activation by INFγ to increase the immunosuppressive capacity of cells and their *in vivo* therapeutic efficacy in mice with colitis induced by trinitrobenzene sulfonate.

MSCs produce a range of factors that have been implicated in immune modulating effects, such as indoleamine 2,3-dioxygenase, IL-6, IL-10, prostaglandin E₂, transforming growth factor-β1, nitric oxide, heme oxygenase-1 and HLA-G5^[109]. Through the production of PGE, MSCs inhibit IL-2 production and T cell proliferation, with stimulation of the production of T helper lymphocytes^[119]. IL-10 and IL-6 act upon macrophages and monocytes, blocking their differentiation towards dendritic cells^[120,121]. They also suppress NK cells, induce

polymorphonuclear cell and cytotoxic T cell apoptosis, and promote the generation of regulatory T cells^[122]. These interleukins also inhibit B lymphocyte activation^[123]. Upon coming into contact with activated T cells, MSCs induce the production of IL-10 and HLA-G5^[124]. In short, they inhibit innate immunity by blocking the differentiation and maturation of monocytes towards dendritic cells, inhibiting NK cells, and inducing neutrophil apoptosis. In induced colitis in mice, MSCs block T cells and increase regulatory T lymphocyte population, thereby modulating acquired immunity.

However, MSCs can acquire proinflammatory properties, depending on biological environment^[125]. In this context, Nemoto *et al*^[126], in a murine model, have shown that bone marrow MSCs constitute the main source of IL-7 production, and may play an etiopathogenic role in IBD by forming a niche for colitogenic CD4⁺ memory T cells in bone marrow. If these findings are confirmed in humans, anti-IL-7 treatment might become a therapeutic target.

MSCs are able to repair tissues, stimulate angiogenesis

and prevent fibrosis. Healing of the intestinal lesions in IBD is the result of control of the underlying inflammatory process, and of repair mechanisms that restore the integrity of the epithelial barrier and repair intestinal damage. The initial applications of MSCs in regenerative medicine were focused on their multilineage differentiation capacity. However, recent reports have demonstrated that most of the biological effects of MSCs are mediated by paracrine mechanisms involving the secretion of cytokines, chemokines and growth factors^[127-129]. Indeed, there are several observations of positive MSC biological effects in numerous disease models despite a lack of MSC differentiation and long-term engraftment into damaged or diseased tissue. These effects include a reduction of inflammation, apoptosis and fibrosis, improved wound healing and regeneration^[130-133]. In addition, MSC biological effects also may be exerted by the induction and stimulation of endogenous host progenitor cells to improve the regenerative process^[134-136].

When administered *via* the systemic route (intraarterial or intravenous) or locally, the objective is to ensure that MSCs reach damaged tissue. However, the correct dose and administration route remain to be defined, and may even differ depending on the type of lesion involved^[69]. Forty-eight hours after systemic administration in animals, the cells are found to be located in the lung and liver^[137], while after 9-21 mo they are detected in the lungs, liver, pancreas, spleen, kidneys and skin^[69,138].

The engraftment rate in these tissues is 0.1%-2.7%, and is similar for both autologous and allogenic MSC transplantation^[138].

Another aspect to be taken into consideration is that the drugs used to treat IBD do not appear to alter MSC function^[139].

MSCs were first used in clinical practice to treat graft-versus-host disease (GVHD) in a patient with acute lymphoid leukemia refractory to therapy. Improvement was notable, and the patient remained free of gastrointestinal lesions after one year^[140].

The company Osiris has created an MSC product based on bone marrow from healthy donors (Prochymal), and has designed a phase II open-label study on the treatment of acute GVHD. Two treatment arms were defined: one group received high-dose MSCs (8 million MSCs/kg), while the other group received low-dose MSCs (2 million MSCs/kg). Two infusions were administered in both groups, spaced three days apart. The following results were obtained: 24 out of 31 patients showed complete clinical response (absence of GVHD symptoms in skin, liver and gastrointestinal tract), while 5 showed partial response^[141]. However, a phase III study involving a similar group of patients failed to obtain significant results in relation to the primary endpoint^[142].

At present, MSCs are being used for repair and immune modulating purposes in clinical trials on the treatment of cardiovascular and neurological disease, in the treatment of autoimmune disorders, and in the management of post-radiotherapy tissue damage^[128,130,132,135,136,143].

Laboratory-based studies reporting antineoplastic activity have recently been published^[144].

Two types of treatment with MSCs have been developed in IBD: systemic administration of MSCs (intraarterial or intravenous) for the control of intestinal inflammatory disease, and the local administration of MSCs in perianal fistulizing CD.

Systemic MSCs therapy in IBD

The use of MSCs for IBD treatment is based on their reparatory and immune modulating properties.

Several phase I - II studies involving autologous and allogenic MSCs have been published in recent years on the treatment of IBD.

In 2006, Onken *et al*^[145] published an abstract on MSCs used to treat 10 patients with active CD (CDAI > 220, C-reactive protein \geq 5 mg/L) refractory to treatment with corticosteroids, immune modulators and infliximab. Use was made of MSCs obtained by bone marrow puncture from healthy donors (Prochymal, Osiris). The patients were randomized to two groups, both of which received two intravenous doses of MSCs, spaced one week apart. One group received high-dose MSCs (8 million MSCs/kg), while the other group received low-dose MSCs (2 million MSCs/kg). The primary endpoint was percentage clinical response, defined as a reduction in the CDAI score of \geq 100 points. Four weeks after the end of treatment, and as a secondary endpoint, improvement was observed in the inflammatory bowel disease questionnaire (IBDQ) scores during the same period. Nine patients completed the study. All subjects presented a mean reduction in CDAI score of 105 points on day 28. The CDAI and IBDQ scores decreased to a greater extent in the high-dose group, though statistical significance was not reached^[145].

A phase III, randomized and placebo-controlled trial was started by Osiris in 2007 (protocol 603, NCT00482092 clinicaltrials.gov)^[146]. The aim was to include a large number of patients with active CD (CDAI score 250-450; PCR \geq 5) and a history of intolerance or resistance to corticosteroids, immune modulators and biological drugs. The patients were randomized to four intravenous MSC infusions in two weeks. One group of patients received a total of 600 million cells (low dose), while the rest received 1200 million cells (high dose) or placebo. The primary endpoint was remission on day 28, while the secondary endpoints were clinical response, improvement in quality of life, and reduction of the number of draining fistulas. In March 2009, with 207 patients enrolled, the trial was suspended because of a high placebo response. Subsequently, the United States Food and Drug Administration authorized the reopening of the study, though no results are currently available^[147].

More recently, phase I studies have been published that allow us to know the safety and possibilities of this type of therapy. In 2010, Duijvestein *et al*^[148] published the results of a phase I trial with intravenous MSCs administered to 10 patients with CD refractory to cortico-

steroids, immune modulators and anti-TNF α drugs. The cells were obtained by bone marrow puncture of CD patients, and 9 subjects received two doses of 1-2 million cells/kg body weight, spaced one week apart. This study showed the autologous MSCs of CD patients to be functionally analogous to those of healthy donors, maintaining the same immune modulating properties, and without experiencing alterations due to the drugs administered to the patient. In relation to CD, the benefits obtained were few: only three patients showed improvement, with a reduction of CDAI ≥ 70 . The procedure proved safe, with no significant side effects.

In 2012, Liang *et al.*^[149] reported the results obtained in 7 patients with IBD (4 with CD and 3 with UC). In three cases MSCs were obtained from the bone marrow of healthy donors, and in four cases from umbilical cord. The dose administered consisted of one million cells/kg *via* the intravenous route. All the patients maintained their medication (corticosteroids and immune modulators) after the infusion of MSCs. Five subjects showed remission, and the latter was maintained for over 24 mo in two of them. Two CD patients and one UC patient showed improved endoscopic indices, and in all three of these subjects biopsies revealed a decrease in the extent of IBD and in intensity of lymphoid infiltrate. Side effects were mild: one patient experienced facial flushing for 6 h after the infusion, while another experienced insomnia during the first night following infusion. One patient developed febricula and worsening of diarrhea. These symptoms disappeared without medication of any kind, and no other side effects were observed.

Regarding the safety of MSC treatment, clinical trials conducted in human subjects have found the therapy to be safe, with no toxic effects or generation of ectopic tissue. The most commonly reported side effect was transient fever^[140,145,146,148-150]. MSCs may be infected with viruses (*e.g.*, cytomegalovirus, herpes virus), and a case of infection with Epstein-Barr virus has been reported in a patient with a lymphoproliferative process previously subjected to bone marrow conditioning treatment, followed by the administration of MSCs due to an episode of GVHD^[151,152]. As mentioned, a possible case of bacterial infection transmitted by umbilical cord hematopoietic stem cells has recently been reported^[62].

The above data are promising, yet the number of treated patients is small, the source of MSCs diverse, and the regimens and doses different. It is therefore not possible to draw firm conclusions at this time. Strictly protocolized trials need to be conducted to draw valid conclusions.

Local MSCs therapy in fistulizing EC

MSCs treatment for perianal CD has been carried out using MSCs obtained from bone marrow or adipose tissue. We do not know exactly the mechanism of how MSCs work in perianal fistulas. As we explained before, MSCs and expanded allogeneic adipose-derived stem cells (ASCs) have the potential to control systemic and local inflammatory pathways by suppressing the proliferation

of activated lymphocytes. By controlling the local inflammatory process they could induce cicatrization of the fistula due to reparative properties.

Regarding the first cell source, the experience gained corresponds only to an Italian study published in 2008^[153]. The authors treated 9 CD patients - 8 with complex perianal fistulas and one with multiple enterocutaneous fistulas - using MSCs from bone marrow, serially injected into the fistular canal every four weeks, with an average of four doses in total. Remission was achieved in 7 of the 10 patients, while improvement was noted in the remaining three, with marked and significant reductions in the perianal disease activity index and CDAI scores. The findings were confirmed by endoscopy and magnetic resonance imaging (MRI), and evident improvement of the rectal inflammation was also evidenced. The authors postulated that part of the effect may have resulted from stem cells reaching the lymph nodes, influencing T lymphocyte differentiation and^[154] apoptosis.

Regarding ASCs, a large number of studies have been published over the last decade. The first such study dates back to 2003^[155]. Based on the previously reported myogenic, adipogenic or chondrogenic differentiation potential of these cells^[156], ASC injection into a recto-vaginal fistula of a CD patient resulted in sealing of the fistula. From this point onwards, a series of studies were conducted to explore the usefulness of these cells in this field. The first publication was a phase I study^[157] carried out performed in 2005 to evaluate the safety and viability of the treatment. ASCs were inoculated in 8 complex fistulas in 5 CD patients, with evaluation of the response after 8 wk. The results showed re-epithelization of the external fistular orifice, with complete sealing of 6 fistulas (75%) and a partial decrease in suppuration in the remaining two (25%). No serious adverse effects were reported. Biopsies obtained from the zone in two patients after 7 and 12 mo showed no evidence of dysplastic transformation.

Following the results obtained, a new phase II, randomized, open-label, controlled multicenter study was published in 2009^[158]. This study included 35 patients with complex cryptogenic fistulas, including 14 associated to CD, randomized to either fibrin glue or fibrin glue plus 20 million ASCs. Evaluations were made after 8 wk and one year, with the possibility of administering a second dose of 40 million ASCs in cases showing failure in week 8. Sealing of the fistula, defined as closure of the external orifice and the absence of suppuration in response to digital pressure, was recorded in 11 patients (46%) in the ASC treatment arm, *vs* in two patients (8%) in the non-cell therapy arm, after 8 wk. In turn, following both doses, sealing was recorded in 17 patients (71%) in the ASC treatment arm and in four patients (16%) in the fibrin glue treatment arm (RR = 4.43, 95%CI: 1.74-11.27, $P < 0.001$). Effectiveness was found to be similar on considering a cryptoglandular origin of the fistulas *vs* fistulas associated to CD, though statistical significance was not reached in this latter subgroup due to the small number

of patients involved. No adverse effects related to ASC use were reported.

The results of the FATTI phase III, randomized, single-blind, multicenter clinical trial were published in 2012^[159]. In this study IBD constituted an exclusion criterion, and a total of 200 subjects diagnosed with simple cryptoglandular fistulas (confirmed by MRI) were included. The patients were randomized to three groups: 20 million ASCs (group A), 20 million ASCs with fibrin glue (group B), and fibrin glue with placebo (group C). The possibility existed of a second dose of 40 million ASCs in week 12. The primary endpoint was fistular sealing (re-epithelization of the external orifice, with absence of drainage and no collections as evidenced by MRI) in week 12 and in weeks 24-26. Of the 200 randomized individuals, 183 received treatment, and 165 completed the study [per protocol (PP) analysis]. After 12 wk fistular sealing was observed in 26.5%, 38.33% and 15.25% of patients in groups A, B and C, respectively ($P = 0.01$). A second dose was administered in 61.5% of patients, with fistular sealing in 39.1%, 43.3% and 37.3%, respectively ($P = 0.79$). Although the findings were not as promising as in previous studies, posterior analysis stratified by center revealed far better results for patients administered ASCs on comparing the center with the greatest experience *vs* the rest of the participating centers: 45.55%, 83.3% and 18.8% ($P = 0.025$ for treatment) *vs* 35.8%, 33.3% and 42.6% for groups A, B and C, respectively. The multivariate analysis moreover showed the fact of having received treatment at the center with most experience to be a significant factor. The authors postulated that the different surgical protocol used in the treatment, as well as the experience of the surgeon in using ASCs in perianal fistulas, may have been decisive. As regards the safety of treatment, there were no significant differences in adverse effects among the three groups. A total of 37 serious adverse effects were recorded - three of which were related to the procedures used, but none to use of ASCs.

In that same year, the first study on donor adipose tissue expanded mesenchymal stem cells (eASCs) was published^[160]. Up until that time, all treatments had been made with autologous MSCs, except in one case where donor cells were used^[161]. This study used an eASC formulation prepared by the company Cellerix (now Tigenix). Twenty-four patients with CD and complex perianal fistulas were enrolled. The primary endpoint was fistular closure in weeks 12 and 24, defined as the absence of suppuration, re-epithelization of the external orifice, and the absence of collections as evidenced by MRI. A starting dose of 20 million cells was injected into the fistula, followed by a second dose of 40 million cells in the event of incomplete closure in week 12. Of the 24 patients included in the study, 16 completed the treatment period and, of these, 69.2% experienced decreased suppuration of at least one of the fistulas, 56.3% showed closure of the treated fistulas, and 30% presented closure of all fistular trajectories. The results were confirmed by MRI. There were two serious adverse effects (fever and perianal

abscess), possibly related to the intervention, although both showed full recovery.

At present, a phase III study is underway involving eASCs for the treatment of complex fistulas in CD patients. This trial will contribute relevant information and may lead to future marketing of the treatment. A new treatment option is thus under development that may revolutionize the management of perianal fistulizing CD. The safety of this cell product appears to have been confirmed by the different studies published to date. Ten years have passed since publication of the first report, and follow-up studies have been made of some of the published series, without the detection of anomalies^[162].

CONCLUSION

At present, treatments with mesenchymal and hematopoietic stem cells offer a potential that requires in-depth investigation. Existing studies are encouraging yet inconclusive. We are at a point of inflexion where these new therapies are seen to afford major curative potential. The coming years will be decisive. The information obtained from ongoing and future clinical trials may lead a revolution in IBD management and its impact upon patients. Undoubtedly, as twenty-first century gastroenterologists, we must expand the scope of our specialty and seek multidisciplinary interaction for the benefit of our patients.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Inflammatory bowel disease: An increased risk factor for neurologic complications

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Abstract

Only a very few systematic studies have investigated the frequency of neurologic disorders in patients with Crohn's disease (CD) and ulcerative colitis (UC), which are the two main types of inflammatory bowel disease (IBD). Results have been inconsistent and variable, owing to differences in case-finding methods and evaluated outcomes in different studies. The most frequent neurologic manifestations reported in CD and UC populations are cerebrovascular disease (with either arterial or venous events), demyelinating central nervous system disease, and peripheral neuropathy (whether axonal or demyelinating); however, the literature describes numerous nervous system disorders as being associated with IBD. The pathogenesis of nervous system tissue involvement in IBD has yet to be elucidated, although it seems to be related to immune mechanisms or prothrombotic states. The recently-introduced tumor necrosis factor (TNF) inhibitors have proven successful in controlling moderate to severe IBD activity. However, severe neurologic disorders associated with TNF inhibitors have been reported, which therefore raises concerns regarding the effect of anti-TNF- α antibodies on the nervous system. Although neurological involvement associated with IBD is rarely

reported, gastroenterologists should be aware of the neurologic manifestations of IBD in order to provide early treatment, which is crucial for preventing major neurologic morbidity.

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Key words: Extraintestinal manifestations; Inflammatory bowel disease; Multiple sclerosis; Neuropathy; Stroke; Tumor necrosis factor inhibitor

Core tip: The neurological manifestations in inflammatory bowel disease (IBD) patients are often unrecognized or underestimated. A detailed revision of the literature about the neurological manifestations in ulcerative colitis and Crohn's disease patients is relevant to the IBD community, especially in the biologics era. Gastroenterologists should be aware of the neurologic manifestations of IBD in order to provide prevention and early treatment, which is crucial for preventing major neurologic morbidity.

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INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the two main types of idiopathic inflammatory bowel disease (IBD), and they are clearly distinct pathophysiological entities. UC, the most common form of IBD worldwide, is a disease of the colonic mucosa only; it is less prone to complications and can be cured with colectomy. In contrast, CD is a transmural disease of the gastrointestinal mucosa which can affect the entire gastrointestinal tract

from the mouth to the anus^[1,2].

CD and UC should be considered systemic diseases since they are associated with clinical manifestations involving organs outside the alimentary tract. Extraintestinal manifestations (EIMs) involving several organs, and most EIMs occurring in the joints, skin, mouth, eyes and coagulation system, either precede the onset of intestinal manifestations or appear and evolve in parallel with them. They also respond to treatment for the underlying bowel disease. However, many EIMs tend to follow a course independent from that of bowel disease activity. These EIMs are observed in some 20%-40% of patients with IBD, with CD patients being more susceptible to EIMs than patients with UC^[3-8].

Neurologic involvement associated with IBD is frequently underreported. Nevertheless, it is important to quantify the morbidity burden of clinically significant neurologic complications in IBD because early recognition and treatment of neurologic diseases are crucial for preventing major morbidity^[9,10]. The available literature consists of case reports and small series and only a few of them have reviewed large groups of IBD patients to identify neurologic symptoms. Moreover, most of the recent reviews dealing with UC and CD include only a brief mention of nervous system involvement in IBD^[2,5].

The pathogenesis of neurogenic disorders associated with IBD has not been established and it may involve diverse causes. Most of them have an immune basis, but other reported causes include prothrombotic states, nutrient deficiency (vitamin B₁₂, folate, copper, thiamine, vitamin E) because of malabsorption, and iatrogenic complications of medical and surgical management of IBD. In addition, in recent years, use of tumor necrosis factor (TNF) inhibitors has emerged as a successful treatment for refractory IBD, although anti-TNF- α antibodies appear to predispose some patients to developing diverse peripheral and central nervous system (CNS) involvement. An approach to neurologic symptoms in patients diagnosed with IBD has recently been reported elsewhere^[11].

Neurologic involvement in IBD as a subgroup of the EIMs may precede the appearance of digestive symptoms or develop after diagnosis of IBD. In addition, neurological symptoms may exacerbate during flare-ups of IBD or evolve independently from intestinal manifestations without responding to treatment provided for the underlying bowel disease^[12,13].

This review will examine current knowledge about nervous system involvement in CD and UC and the neurologic manifestations secondary to the use of biological agents and other approaches to IBD management.

EPIDEMIOLOGY OF NEUROLOGICAL MANIFESTATIONS OF IBD

Few systematic studies have investigated the frequency of neurological disorders in patients with IBD. Additionally, results from these studies have been inconsistent, which

is mainly due to discrepancies in case-finding methods. In most of the studies reported, magnetic resonance imaging was not part of the standard workup. Moreover, some studies have included neurological symptoms of iatrogenic origin or symptoms caused by malabsorption-related disorders secondary to vitamin deficiencies^[14,15]. Most of these studies were retrospective and register-based, and only a few included a control group^[16]. Finally, the latest reports on neurologic complications in IBD disease have focused on peripheral nervous system (PNS) involvement, and they lack data on clinical manifestations involving other nervous system components^[14,17-20]. Table 1 presents clinical studies addressing the frequency of neurogenic disorders in CD and UC. In a large retrospective register-based study including 638 patients with UC or CD, Lossos *et al*^[21] found neurological involvement in 3% of the cases. In contrast, 33.2% of patients in a CD population experienced neurological or neuropsychiatric complications, although this proportion decreased to 19.3% when considering only those cases with a direct relationship^[15]. Finally, in another study, 67% of patients with CD and 53% of patients with UC had neurologic disorders, although authors did not report whether IBD and neurologic involvement were coincidental^[19]. Table 2 lists neurological disorders reported in the literature as being associated with IBD.

NON-DRUG-INDUCED NEUROLOGICAL MANIFESTATIONS OF IBD

Peripheral neuropathy

Peripheral neuropathy (PN) is known to be related to IBD and it is one of the most frequently reported neurologic complications. Various studies have found PNS complications, rather than CNS involvement, to be predominant^[14]. Initially, medical treatments for the gastrointestinal disease or vitamin deficiencies caused by malabsorption were thought to cause PN^[21]. Ultimately, PN is a common adverse event associated with use of TNF inhibitors^[22]. If these causes of neuropathy are excluded, however, the reported frequency of PN in IBD will vary greatly among published studies, with estimates ranging from 0% to 39% due to selection bias, use of different definitions of the disease, or population characteristics^[14,15,18,20,21,23].

Two recent studies have indicated the low prevalence of PN in IBD. In the first study, carried out in Greece, 97 patients with any form of IBD were studied to identify any cases of PN. Fifty-two patients were excluded for different reasons, including presence of comorbidities associated with PN and presence of other neurologic symptoms. Nerve conduction studies in the remaining 45 asymptomatic patients yielded normal results except for one patient with a history of acute motor sensory polyneuropathy complicating UC and another patient with incidental carpal tunnel syndrome^[17]. A second study in Olmsted County, Minnesota, determined the neuropathy incidence rate in a population-based cohort of patients

Table 1 Clinical studies of neurologic disorders in inflammatory bowel disease

Author	Year	Country	Study design	Total patients	Type of IBD	Patients with NC	Age (range, yr)	Sex	Other EIM	Associated with IBD exacerbation	Incidence of NC
Lossos <i>et al</i> ^[21]	1995	Israel	Retrospective, computerized search	638	377 CD 261 UC	10 CD patients 9 UC patients	11-71	11 M 8 F	50%	2 patients (10%)	19 patients (3%)
Elsehety <i>et al</i> ^[15]	1997	United States	Retrospective	253	CD	Probable: 49 patients Possible: 35 patients Total: 84 patients	NR	NR	NR	5 patients (6%)	Probable: 19.3% Possible: 13.9% Total: 33.2%
Oliveira <i>et al</i> ^[14]	2008	Brazil	Prospectively, clinic-based, PN especially studied	82	31 CD 51 UC	16 CD patients 23 UC patients	43 CD ¹ 51 UC	15 M 24 F	NR	NR	PN: 51.6% CD 45.1% UC Headache: 54.8% CD 57% UC 8.80%
Sassi <i>et al</i> ^[23]	2009	Tunis	Prospective, prevalence study PN	102	88 CD 14 UC	6 UC patients ² 3 CD patients	22-64	7 F 2 M	33%	29%	30%
Benavente <i>et al</i> ^[12]	2011	Spain	Retrospective, hospitalized patients	84	NR	13 UC patients 12 CD patients	17-74	12 M 13 F	4%	10 patients (40%)	38.70%
Shen <i>et al</i> ^[20]	2012	United States	Questionnaire-based, study of PN symptoms only	173	102 CD 71 UC	67 patients	NR	NR	NR	NR	72 patients/100000 person-years
Figueroa <i>et al</i> ^[18]	2013	United States	Retrospective, observational population based-cohort	772	342 CD 430 UC	9 patients/12 events 6 UC patients 3 CD patients	21-83	5 F 4 M	NR	8 patients	No patients had subclinical neuropathy
Babali <i>et al</i> ^[17]	2013	Greece	Prospective study of subclinical PN in asymptomatic patients	45 ³	30 CD 15 UC	0 patients	NR	NR	NR	NR	

¹Mean age; ²Three patients had mononeuropathies due to rapid weight loss. Three patients had sensory large-fiber polyneuropathy and two had small-fiber polyneuropathy. One patient had a sensorimotor axonal PN. An etiological factor was identified in four cases (diabetes mellitus and long-term metronidazole treatment); ³Two patients were excluded: one had history of acute motor sensory polyneuropathy complicating UC and other patient had incidental carpal tunnel syndrome. IBD: Inflammatory bowel disease; NC: Neurologic complications; EIM: Extraintestinal complications; CD: Crohn's disease; UC: Ulcerative colitis; F: Female; M: Male; NR: Not reported; PN: Peripheral neuropathy.

newly diagnosed with IBD over a 64-year period. The overall incidence rate was 72 cases of neuropathy per 100000 IBD person-years with a cumulative incidence rate after 30 years of 2.4%. Moreover, the study found late occurrences of neuropathy in the course of IBD, mainly during IBD inactivity^[18].

Regarding the type of neuropathy reported, studies describe demyelinating or axonal involvement of peripheral nerves in IBD, and both neuropathies may be acute or chronic. Several cases of immune-mediated neuropathies in IBD patients have also been reported^[24-26]. In a retrospective review of patients with PN and IBD, more than two-thirds of the cases had axonal neuropathy. Neuropathies were predominantly sensory (either small-fiber or large-fiber) rather than sensorimotor for axonal neuropathies; only one-third of patients developed demyelinating forms of neuropathy^[19]. Conversely, the clinical spectrum reported by Figueroa *et al*^[18] consisted of monophasic immune radiculoplexus neuropathy and

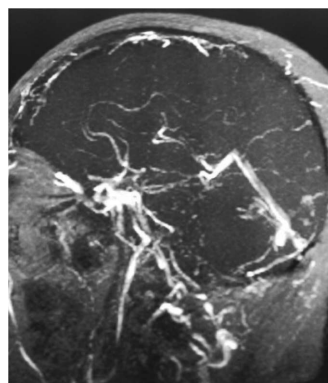
chronic distal sensorimotor polyneuropathy. The underlying pathophysiology in cases of neuropathy in IBD patients remains obscure. T-cells are clearly involved in the pathogenesis of demyelinating neuropathies. At the same time, the relationship between axonal damage and immune system disturbances remains unclear, but clinical improvement in patients treated with immunomodulatory agents suggests that there is a link^[19].

Cerebrovascular disease

Thromboembolic complications are two to four times more likely in patients with IBD than in healthy individuals; they occur at any age in both sexes and active IBD may increase the relative risk of such complications by as much as 15-fold^[27]. The incidence of thromboembolism in IBD ranges between 1% and 7.7% in clinical studies, with postmortem studies reporting rates of 40%^[28]. Deep vein thrombosis and pulmonary embolism are the most common entities, but cerebrovascular disorders also oc-

Table 2 Neurologic disorders associated with inflammatory bowel disease reported in the literature

Cerebrovascular disease
Cerebral infarction
Transient brain ischemia
Cerebral venous thrombosis
Demyelinating disease
Multiple sclerosis
Asymptomatic focal white-matter lesions
Myelopathy
Optic neuritis
Inflammatory pseudotumor
Epilepsy
Seizures
Psychosis
Chorea
Major depression
Autonomic nervous system dysfunction
Vasculitis of the central nervous system
Restless legs syndrome
Sleep disruption
Headache
Cranial neuropathies
Melkersson-Rosenthal syndrome
Sensorineural hearing loss
Ischemic optic neuropathy
Bell's palsy
Neuromuscular diseases
Myasthenia gravis
Myopathy
Dermatomyositis
Polymyositis
Vacuolar myopathy
Peripheral neuropathy
Sensory large-fiber polyneuropathy
Small-fiber polyneuropathy
Acute and chronic immune-mediated neuropathies
Monophasic immune radiculoplexus neuropathy
Chronic distal sensorimotor polyneuropathy
Mononeuritis multiplex

**Figure 1** Sagittal magnetic resonance venography imaging scan showing thrombosis of the superior sagittal sinus in a 51-year-old man with cerebral venous thrombosis. He had been diagnosed with ulcerative colitis involving the ileum and colon 15 years before.

cur and they are probably underestimated^[29]. In a meta-analysis published in 2013, CD and UC were found to be associated with an increase in the risk of ischemic or hemorrhagic stroke or transient ischemic attack (OR = 1.28, 95%CI: 1.17-1.41), especially among women and young patients^[30]. Strokes may affect either arterial or venous territories, but it is unclear whether venous or arterial strokes are more frequent^[31,32].

The literature describes cerebral infarction and transient brain ischemia due to small- and large-artery disease involving the anterior and posterior circulation. Carotid arterial thrombosis, retinal branch artery occlusion, carotid thromboembolism, cardiac embolism, and paradoxical embolism are other possible pathogenesis^[33-36].

IBD accounts for 1%-6% of the total causes of cerebral venous thrombosis (CVT), and CVT develops without there being a temporal relationship with the course of IBD (Figure 1). There are no appreciable differences between IBD-related CVT and non-IBD related CVT in terms of clinical or radiological characteristics, prognosis, or treatment^[37-39].

The reason for the increased rate of thromboembolic

events in patients with IBD remains uncertain, but is most likely related to the interaction between acquired and genetic risk factors. Researchers have also reported interaction between cytokine mediators of chronic inflammation and the coagulation cascade^[40].

Some genetic factors listed as possible promoters of the thrombotic manifestations of IBD include factor V Leiden and factor II, methylenetetrahydrofolate reductase gene mutation, plasminogen activator inhibitor type 1 gene mutation, and factor XIII^[28]. However, no studies have convincingly demonstrated that IBD patients have a greater burden of prothrombotic genetic or non-genetic risk factors, such as factor V Leiden mutations, hyperhomocysteinemia, antiphospholipid antibodies, or thrombophilia, than the general population^[41-43]. Moreover, genetic risk factors are generally not found more often in IBD patients than in the general population. However, when such factors are present, patients with IBD are more likely than healthy controls to suffer thromboembolic complications^[28].

In addition to platelet and endothelial activation, some of the most often-cited impaired coagulation states are high levels of factor V, factor VIII, fibrinogen, and von Willebrand factor. In addition, low activity and low concentration of XIII factor subunit A have been detected. This decreased activity inversely correlates with intestinal inflammatory activity and fibrinogen levels^[44]. In addition, IBD patients display decreased levels of protein C, protein S, antithrombin III, and tissue factor pathway inhibitor (all of which function as anticoagulants), and decreased levels of tissue plasminogen activator with increased plasminogen activator inhibitor-1; the above are all key players in the fibrinolysis pathway^[36]. Finally, other mechanisms that may explain cerebrovascular disorders in IBD include vasculitis and consumption coagulopathy leading to hemorrhagic events^[21]. Based on these data, recent studies have suggested that IBD may be an independent risk factor for thromboembolic disorders^[27,36,45].

Demyelinating disease

The relationship between multiple sclerosis (MS) and

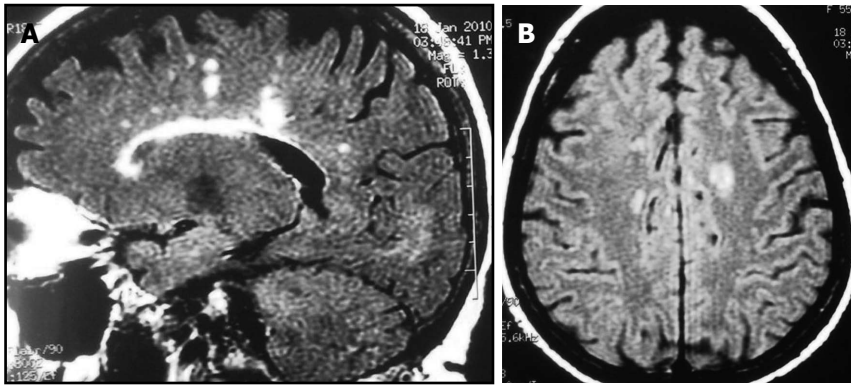


Figure 2 Sagittal (A) and axial (B) cranial magnetic resonance imaging scan depicting several hyperintense focal lesions in supratentorial white matter. The patient was a 45-year-old woman with relapsing-remitting multiple sclerosis and a 23-year history of ulcerative colitis.

IBD was discovered by Rang *et al.*^[46] in the early 1980s when they detected high MS incidence and prevalence while studying patients who had undergone a colectomy for IBD (Figure 2). Retrospective cross-sectional studies have reported an increased incidence of demyelinating disease^[16]; however, incidence of MS was reported to be higher in patients with UC than with CD^[47]. In addition, one study found a high prevalence of asymptomatic focal white-matter brain lesions in IBD patients^[48] but these data not have been confirmed by more recent studies^[49]. Moreover, the incidence of white matter hyperintensities on T2-weighted images was found to be higher in CD patients (72%) than in age-matched controls (34%); however, this population was not completely asymptomatic and 20% of the CD patients were on TNF inhibitors.

Several studies have documented additional autoimmune diseases, including IBD, in patients with MS and their relatives; however, results were disparate due to differences in study design^[50,51]. In a Danish study, Nielsen *et al.*^[52] found that MS patients were at a higher risk for developing UC and other autoimmune diseases, but not CD. This hypothesis is supported by a recent systematic review and meta-analysis. Here, the authors reported a significant relationship between MS and IBD (OR = 1.56, 95%CI: 1.28-1.90), although this association was not found in relatives of MS patients^[53].

There may be a significant relationship between MS and IBD. This possibility is supported by the role played by immune mechanisms in the pathogenesis of both disorders and by fact that immune system inhibitors that impair cell-mediated immunity are effective for treating both MS and IBD. Disturbances in functional T-cell subsets as well as in antigen-presenting cells have also been implicated. Studies suggest that aberrant proinflammatory activity in particular may be a common pathway leading to the destruction of target tissue in both diseases^[3-5].

Miscellaneous

Studies have reported an association between epilepsy and IBD; however, the possible relationship between epilepsy and IBD has not been completely addressed^[12,15,21]. Furthermore, seizures may be symptoms of systemic

or brain processes such as hypomagnesemia or cerebral venous thrombosis^[54,55]. Vasculitis of the CNS and transverse myelitis associated with Jo-1 antibody syndrome are additional complications that have been described in IBD patients^[56-58].

In a prospective multicenter study in patients with CD, incidence of restless legs syndrome (RLS) was 43% and prevalence was 30%. This prevalence rate exceeds that of the general population, and the incidence figure is higher than the incidence of many known EIMs of CD. Furthermore, RLS symptoms occurred during or after the onset of CD symptoms in most patients, suggesting a link between CD and RLS^[59]. Recent studies have shown that sleep disorders are more common in IBD patients, and treating sleep disruption with a melatonin supplement has been shown to improve sleep in animal models of immune colitis^[60].

Some authors suggest that the autonomic nervous system plays a role in the pathogenesis of IBD and that IBD may be a potential neurologic complication of chronic inflammation. On the other hand, autonomic dysfunctions have been reported in patients with IBD; although earlier reports suggested autonomic hypofunction, later studies also identified autonomic hyperreflexia. Sympathetic autonomic neuropathy is an early systemic feature of CD and one that is independent from disease activity. Moreover, clinically manifest autonomic dysfunction is associated with lower quality of life and more intensive healthcare use in IBD patients^[61-63].

Sensorineural hearing loss (SNHL), first described in 1982, is currently a well-recognized EIM of UC^[64]. In a study of 38 patients with a history of IBD, 59% had inner ear dysfunction that was probably related to gastrointestinal disease. Of the 19 patients with hearing loss (14 patients had UC and 5 had CD), 16 patients had bilateral SNHL; unfortunately, only one patient experienced clinical improvement following medical treatment^[65]. The pathogenesis of SNHL associated with UC is not fully understood, and several immune mechanisms may lead to inner ear dysfunction^[66]. Bilateral anterior optic neuritis and ischemic optic neuropathy due to UC and CD have been described^[67,68]. Lastly, researchers have reported pe-

ripheral facial palsy and Melkersson-Rosenthal syndrome associated with CD^[69].

Furthermore, myasthenia gravis (MG), whether in its ocular or generalized form, has been reported in association with both UC and CD. Intriguingly, two case reports of patients with MG and IBD describe clinical improvement of neuromuscular disease and IBD following different surgical procedures, one after thymectomy and the other following proctocolectomy. These reports emphasize the immunological link between MG and IBD^[21,70-74]. In addition to MG, cases of IBD associated with polymyositis or dermatomyositis have also been described^[75-77].

DRUG-INDUCED NEUROLOGIC MANIFESTATIONS OF IBD

Tumor necrosis factor inhibitor therapy

TNF inhibitors are currently approved as treatment for IBD, including both CD and UC. The proinflammatory cytokine TNF- α has been identified as playing a pivotal role in the inflammatory cascade that causes chronic intestinal inflammation in IBD. Synthetic anti-TNF- α antibodies have been shown to mitigate this inflammatory process. Infliximab was the first TNF inhibitor successfully used to treat IBD. Other drugs with demonstrated efficacy as IBD treatments include adalimumab and golimumab (humanized monoclonal antibodies), and certolizumab pegol (humanized anti-TNF- α antibody Fab' fragment conjugated with a polyethylene glycol molecule). Conversely, etanercept (non-antibody soluble recombinant TNF receptor-Fc fusion protein) is not an effective treatment for IBD^[78].

Since TNF inhibitors were first used in 1996^[79], researchers have reported an increased risk of CNS events (new onset or exacerbation of demyelinating events, including optic neuritis and MS); in addition, PNs have also been reported in small clinical series. Information regarding management and clinical significance of such events is very limited because the incidence of neurologic events is estimated at less than 1 event per 1000 patients during TNF-inhibitor treatment^[78,80-82].

The FDA's most recent report on adverse events related to TNF- α inhibitors from dates to December 31, 2009. Here, the second-highest number of reported adverse events, after patients with rheumatoid arthritis, was in patients with IBD (140 cases, 18.1% of the total reports). These figures show that IBD may be a risk factor for neurologic disease. Most of the cases involving infliximab and adalimumab are linked to PN (42%), followed by CNS or spinal demyelination (17.2%), optic neuritis (17.4%) and facial palsy (7.8%). In addition, authors reported 19 patients diagnosed with transverse myelitis, leukoencephalopathy, unspecified demyelinating disease, encephalopathy, meningitis due to *Listeria*, or right cerebellar neuroglial cyst. Last of all, one case of PN and another case of CNS demyelination associated with certolizumab (17.2%) have also been reported^[22]. These data are consistent with results from the BIOE-

GAS registry of the Spanish Society of Internal Medicine which reported a total of 175 cases of central demyelinating processes and 44 cases of PNs resulting from TNF-inhibitor therapy as of July 2009^[83]. Recent studies have highlighted the development of posterior reversible encephalopathy syndrome in pediatric patients with IBD treated with infliximab^[84]. Although several hypotheses have been proposed in an attempt to explain the possible relationship between TNF inhibitor treatment and CNS events, none is considered to be completely satisfactory. Some authors propose that systemic administration of TNF- α inhibitors may enhance different functions of the immune system which are known to activate demyelinating processes^[85-87]. However, other authors prefer to seek genetic connections, such as haplotypes of TNF- α or signal transducer and activator of transcription 3 (STAT3), which could explain the emergence of neurologic events in IBD patients treated with TNF inhibitors^[88-90].

Regarding PNS disease, most reported types of neuropathy involve demyelination. Cases of Guillain-Barré syndrome account for the majority, but cases of multifocal motor neuropathy with conduction block, chronic inflammatory demyelinating polyradiculoneuropathy, and Lewis-Sumner syndrome have also been reported. Moreover, mononeuropathy multiplex and axonal sensorimotor polyneuropathies have been described, which adds an axonal process to the physiopathology of nervous system damage elicited by anti-TNF agents^[83,91,92]. The proposed pathogenesis of PNs associated with TNF inhibitors includes a T-cell and humoral immune attack against peripheral nerve myelin, vasculitis-induced nerve ischemia, and inhibition of signaling support for axons^[91].

Prognosis in cases of TNF inhibitor-induced neurologic events is usually good if the treatment is discontinued. Gastroenterologists should therefore be mindful of the neurologic complications of biological treatments in order to recognize them immediately; neurological consultations may play a key role in the assessment of these patients^[11,22]. In light of the data reported here, use of TNF inhibitors should be avoided in patients diagnosed with PN or MS.

Other biological agents

Natalizumab is an IgG4/ κ humanized monoclonal antibody which interferes with the interaction between very late antigen-4, expressed on leukocytes, and vascular adhesion molecule-1, expressed on endothelial cells, thus preventing leukocyte extravasation in inflamed sites^[93]. Since natalizumab's mechanism of action differs from that of other biological agents, it represents an important therapeutic option for CD patients who cannot tolerate other treatments or who are experiencing decreased treatment efficacy, particularly when TNF inhibitor treatment does not achieve remission.

The main neurologic adverse event in patients with CD treated with natalizumab is progressive multifocal leukoencephalopathy (PML). The incidence reported in these patients is 2.13 out of 1000 patients. However,

it seems possible to stratify risk of PML among natalizumab-treated patients given that risk mainly tends to be higher in cases of more than 2 years of natalizumab therapy, positivity for anti-JC virus antibodies, and combined use of natalizumab and immunosuppressive drugs^[94]. No cases of PML were reported in three recent reports including 154 CD patients on natalizumab^[95-97].

Non-biological agents

Cyclosporine is a well-recognized treatment for acute severe UC. Between 10% and 28% of the patients on cyclosporine develop neurotoxicity. The most common associated CNS disorder is a postural tremor that affects the upper extremities and responds to beta-blockers. Severe symptoms affect up to 5% of patients and include psychoses, hallucinations, blindness, seizures, cerebellar ataxia, motor weakness and leukoencephalopathy^[98-100].

A pure sensory or autonomic neuropathy is a well-known PNS complication following metronidazole treatment. The neuropathy usually resolves completely once the antibiotic is discontinued^[101].

Sulfasalazine (sulphasalazopyrine) is prescribed as treatment for IBD due to its immunomodulatory action. Reported serious adverse reactions include transverse myelitis and encephalopathy^[102,103].

Finally, it is well known that psychiatric symptoms can develop in association with administration of corticosteroids in IBD patients, although some authors have reported unusual cases of psychotic episodes in UC and CD patients who were not receiving steroids^[104-108].

In conclusion, reports about the incidence and type of neurologic diseases associated with CD and UC are controversial. Therefore, prospective studies are needed to clarify these points, as well as basic studies addressing the multiple mechanisms responsible for nervous system damage.

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Epidemiological studies of migration and environmental risk factors in the inflammatory bowel diseases

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Abstract

Inflammatory bowel diseases (IBD) are idiopathic chronic diseases of the gastrointestinal tract well known to be associated with both genetic and environmental risk factors. Permissive genotypes may manifest into clinical phenotypes under certain environmental influences and these may be best studied from migratory studies. Exploring differences between first and second generation migrants may further highlight the contribution of environmental factors towards the development of IBD. There are few opportunities that have been offered so far. We aim to review the available migration studies on IBD, evaluate the known environmental factors associated with IBD, and explore modern migration patterns to identify new opportunities and candidate migrant groups in IBD migration research.

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Epidemiology; Risk factor; Envi-

ronment; Hygiene hypothesis

Core tip: Inflammatory bowel diseases (IBD) are well known to involve genetic and environmental risk factors. Cohorts from low IBD prevalence regions migrating to areas of high IBD prevalence are candidates to assess further environmental factors - both protective and promotional. There are few opportunities to identify migratory populations to highlight these effects of environmental risk factors in the development of IBD and to compare first- and second-generation migrants. This review highlights known migratory cohorts and identifies an emerging cohort in Middle Eastern migrants to Australia.

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INTRODUCTION

Inflammatory bowel diseases (IBD) are idiopathic chronic diseases of the gastrointestinal tract well known to be associated with both genetic and environmental risk factors. With increasingly new and powerful genetic techniques, it is worthwhile to re-evaluate the contribution of the environment to the development of both Crohn's disease (CD) and ulcerative colitis (UC). To do this, epidemiological research is the key, and such studies have already identified the increasing global incidence and prevalence of IBD in developing countries as these countries become more developed and "Westernised", highlighting the significance of environmental factors on influencing IBD development globally^[1]. However, despite the no-

table amount of studies already conducted on this issue, many of the suggested environmental risk factors for CD and UC still remain contentious in regards to their exact relationship with the diseases^[2].

Currently, only smoking has been an established risk factor for CD^[3] but paradoxically is protective for UC as found by several studies^[4-6]. There seems to be a dose-response relationship between smoking and IBD^[7] and childhood exposure to tobacco smoke is associated with a higher risk of developing CD^[8]. A meta-analysis found that oral contraceptive pill (OCP) increased the risk of both CD and UC, with a dose-response relationship for CD, and OCP discontinuation reduced IBD risk^[9]. Appendectomies are found to have an inverse relationship with UC^[10]. For CD, some suggest insignificant association with appendectomies^[11,12], while others demonstrate increased risk^[12-15]. A meta-analysis found breastfeeding to be protective for both CD and UC^[16]. However, this result is not reproducible by several studies^[17,18]. Generally, antibiotics are found to be a risk factor for CD^[18-20], and a case-control study has found a positive association between CD and UC^[21]. Regarding diet, the literature is inconsistent and no clear dietary risk factors for CD and UC can be determined^[2]. Furthermore, there are also investigations into “proxy measures” of the hygiene hypothesis, including family size and rural dwelling. The hygiene hypothesis has notable popularity as it fits the observation that incidence and prevalence of autoimmune diseases including IBD increase in nations with improvements in hygiene as a result of modernisation^[22]. Improved hygiene may alter intestinal commensal bacteria colonisation, reduce exposure to infectious diseases and vaccinations, which are all speculated to play a role in IBD pathogenesis^[23]. However, many studies investigating factors related to hygiene have conflicting results^[24]. Overall, the epidemiological studies on IBD environmental risk factors have produced important but inconsistent findings, and methodological alterations in future studies are worthwhile to be considered.

We propose a different method to approach epidemiological research on IBD environmental risk factors: using migrant groups as the study population of epidemiological studies. Groups migrating from areas of low IBD incidence to areas of high IBD incidence will provide insight into the effects of environmental triggers on disease development better than populations without such a transition in environment due to the shortened interval between risk factor exposure and IBD onset. Furthermore, studying the offspring of migrants will provide new cohort data on generational changes and their relationship with IBD development and highlight the contribution of these environmental factors at different ages. We aim to review the available migration studies on IBD and explore modern migration patterns to identify new opportunities in IBD migration research. In particular, we propose that a suitable migrant group for environmental risk factor studies is the migrant population from the Middle East to a developed country such as Australia.

SEARCHING

A comprehensive search on the Ovid MEDLINE database (1946 to present) was performed using the following keywords: “inflammatory bowel disease”, “CD”, “UC”, “epidemiology”, “incidence”, “prevalence”, “clinical characteristics”, “extraintestinal manifestations”, “risk factors” and “migrant”. The search was limited to the English language. A manual search of reference lists of all original articles retrieved was also conducted. For migrant studies, 3 were selected after excluding studies which did not investigate at least two generations within migrant groups. To study potential new migration groups that might provide new IBD research opportunities, we searched for epidemiological studies relating to populations of Middle Eastern heritage and their immigration to Australia. The search terms “Middle East”, “Arabic” and “Australia” were used and there were a total of 23 IBD articles pertaining to the Middle East and 2 pertaining to Australia. Figure 1 discloses the literature search, included and excluded articles.

RESEARCH

Advantages of migrant studies

Studies of migrant populations identify disease characteristics of a group in their original location, and trace the group through several generations in the new host country to provide deeper insights into associations between environmental factors and disease expression. Migrant populations have been identified as the most promising model for investigating the changing epidemiology of IBD previously^[25]. A criticism of prior risk factor studies is the long time interval between presumed exposure to the environmental trigger and disease development^[26], creating uncertainty for etiology. Migrant studies are able to overcome this since migrant cohorts transitioning from developing to developed countries offer a shorter time period between risk factor exposure and disease onset, and many risk factors are postulated to be associated with the lifestyle of developed, Westernised countries. Another advantage is that as immigrants adopt new lifestyles, investigating disease characteristics in successive generations will demonstrate temporal relationships between environmental triggers and development of disease, provided that intermarriage with natives is uncommon^[27]. Migrant studies also have the added advantage of overcoming data incompatibility that often occurs when comparing epidemiological data of an ethnic group from a developing country to a similar ethnic group in developed nations due to inequality of diagnostic facilities and healthcare access.

Existing migrant studies

In Leicestershire, United Kingdom, a retrospective cohort study compared the incidence of UC in first and second generation South Asian immigrants to Europeans using both hospital and general practice sources. Stan-

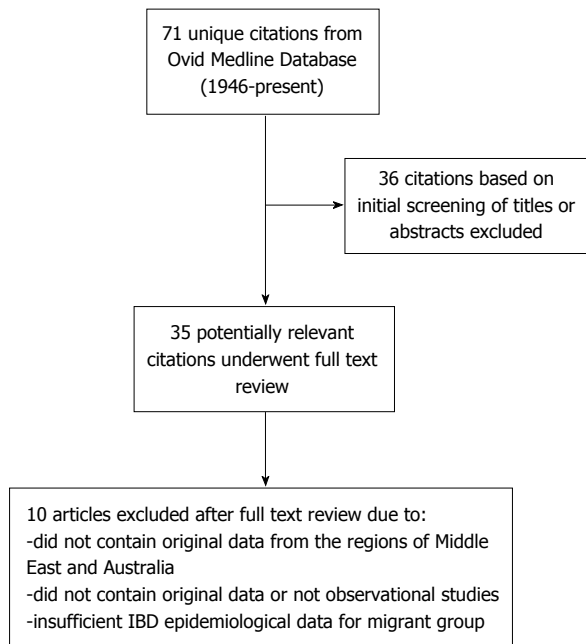


Figure 1 Included and excluded articles. IBD: Inflammatory bowel diseases.

standardised UC incidences for Europeans and South Asian migrants were 5.3 per 10^5 and 10 per 10^5 respectively. Additionally, disease distribution was similar between the two groups, while bimodal age distribution of UC is evident in the European population but not shared by the South Asian migrants. Although unable to accurately determine UC incidence in the two generations separately, the study estimated that first and second generation South Asians are likely at equal risk in developing UC using age-standardised incidences^[28,29]. However, a later prospective multicentre study using hospital-based data also conducted in Leicestershire found higher UC incidences for both the Europeans and South Asian first and second generation immigrants (7.0 per 10^5 and 17.2 per 10^5 respectively). Importantly, second generation South Asians were found to suffer higher rates of extensive colitis compared to the first, and were similar to the Europeans^[30]. The findings from these two studies challenge earlier Asian epidemiological studies^[28,31-42] which reported lower UC incidences, and that environmental impacts cause more severe disease phenotype in later generations. This strongly supports the influential role of environmental risk factors on IBD, which is best investigated by migrant studies comparing successive generations.

Another notable epidemiological study focusing on migrant populations was conducted in Sweden and used a well-defined open cohort study of first and second generation immigrants from 19 countries. The overall IBD risk compared to native-born Swedes was found to be decreased for all first generation immigrants (standard incidence ratios: CD 0.75 per 10^5 , UC 0.81 per 10^5). However, when separated into individual countries, several migrant groups originating from developing countries showed increased risk (Figure 2). The highest standardised incidence ratio in second generation immi-

grants for developing CD were those with one parent or two parents born in Iraq (1.85 and 1.99 respectively), and for UC were immigrants with one (1.55) or both parents (1.68) born in Iran^[43]. These results not only suggest that environmental risk factors have varying effect on risk of IBD depending on ethnicity, but also confirm that migrants moving from developing to developed countries may be most susceptible in demonstrating the effects of IBD development due to environmental triggers. Furthermore, the study identified that one of the region which contained the greatest rise of IBD incidence in migrants as the Middle East (Lebanon, Iraq and Iran). Despite the lack of confirmed case ascertainment, this nationwide study is the largest study to date which has investigated generational IBD epidemiological patterns for a large range of migrants from developed and developing countries (Figure 2).

Currently, many studies which compare different ethnic groups within developed and developing countries exist^[38,44-48]. These studies are able to characterise ethnic differences in IBD, but for purposes of identifying novel risk factors in IBD expression for particular ethnic groups, migrant studies tracing generational changes are superior since genetic variables are controlled. The studies from Leicestershire and Sweden highlight that generational investigations show highly significant IBD epidemiological differences that are most likely due to environmental causes. Unfortunately, the studies have only investigated incidence and prevalence rates. Future studies should additionally investigate risk factor profiles in successive generations. Furthermore, exploring generational changes are important for understanding the role of family history in IBD expression. A study in Leicestershire found the risk of developing UC was 15-fold for first degree relatives of European patients and 3.5-fold for South Asians. However, prevalence in siblings was comparable between the two ethnicities^[48]. Examining generational alterations will shed greater light on the role of inheritance versus environmental contribution towards the development of IBD.

When considering the results of migrant studies, one must be aware of their limitations. Health seeking behaviour and health literacy may be lower in migrant groups, hence reducing IBD detection. If this is the case then any increase in incidence and prevalence reported in migrants in developed countries may be even higher than recorded. Generational environmental changes may also mask the absolute risk of developing CD or UC in immigrants^[43]. For example Swedish Iranian immigrants have higher smoking rates than Iranians in Iran^[49], which may reduce UC incidence for the immigrants as smoking is protective. These examples would reduce the incidence of IBD in migrants. Therefore results that demonstrate an increase in IBD incidence are likely to be of importance. Finally, it is also important to remember that when interpreting risk factor studies, ethnic factors such as diet may be difficult to differentiate from true environmental influences in epidemiological studies^[26]. This may affect

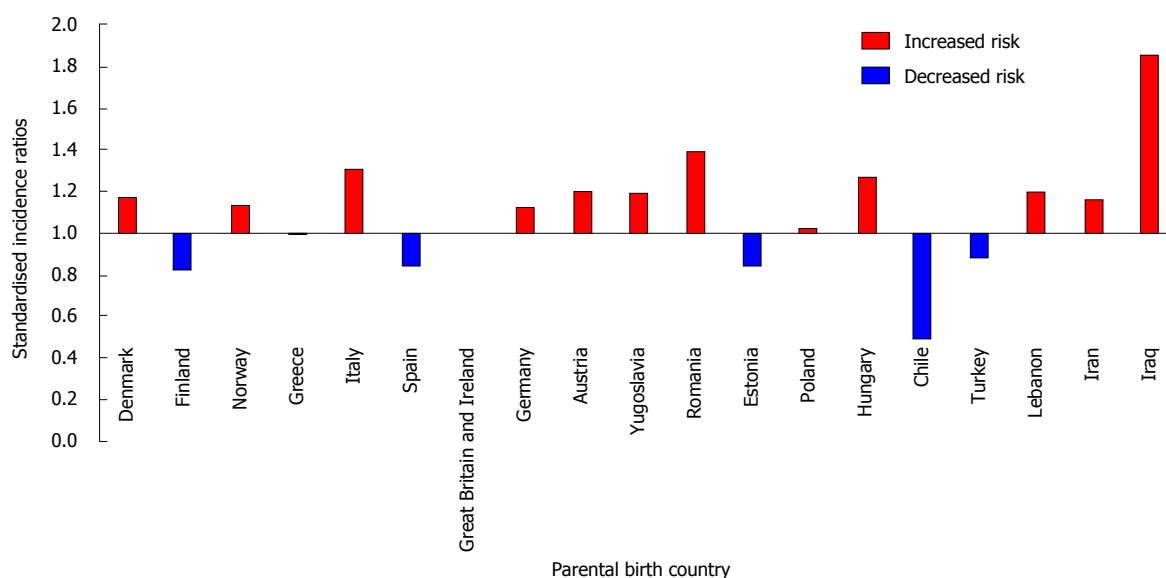


Figure 2 Summary of standardized incidence ratio for Crohn's disease and ulcerative colitis in second-generation immigrants (adapted from ref. [43]).

migrant studies which focus on specific ethnic groups. One way to minimise these confounders is to use controls of the same ethnicity and socio-cultural background.

In the light of the findings of previous migrant studies and modern migration patterns, we aim to identify appropriate migrant groups for future epidemiological research on the risk factors of IBD. Ideally, candidate groups should have good quality epidemiological data on IBD in the country of origin and in the host country, particularly in confirming low IBD incidence rates in the country of origin and high IBD incidence rates in the host country. We propose that migrants from the Middle East migrating to developed countries, such as Australia, are suitable candidates for such studies.

EPIDEMIOLOGY OF IBD IN THE MIDDLE EAST

There is a relatively significant amount of epidemiological research in certain Middle Eastern countries, considering the developing nature of this region. Overall there are 20 incidence^[31-34,37,38,47,50-61] and 19 prevalence^[31,32,35,36,50,51,53,55,56,58,60,62-65] studies on CD and UC in the Middle East from the countries of Lebanon, Israel, Kuwait, Turkey and Oman. Tables 1 and 2 demonstrate the incidence and prevalence findings of these studies stratified by CD and UC, including references for each specific study. Eleven studies^[52-58,64-67] had case ascertainment of UC and CD using the internationally accepted Lennard-Jones criteria, and two were prospective cohort studies^[56,59].

Incidence and prevalence

In the Middle East, annual incidence rates for CD range from 1.1 per 10⁵ in Beer Sheva^[36] to 5.0 per 10⁵ in the Kibbutz settlements in Israel^[55], while ranges were from 1.35 per 10⁵ in Oman^[59] to 6.3 per 10⁵ in Jerusalem in Israel^[37] for UC. The prevalence rates for CD range from

3.2 per 10⁵ in the Bedouin Arabic population in Southern Israel^[62], and 67.9 per 10⁵ in Kibbutz settlements in Israel^[64], while ranges were from 4.9 per 10⁵ in Turkey^[58] to 168.3 per 10⁵ in the Kibbutz settlements in Israel for UC^[66]. Such data indicate that IBD incidence and prevalence are up to 20-fold lower than other locations in the world. In Europe, North America and Australia the highest UC incidence rates of 24.3 per 10⁵, 19.2 per 10⁵ and 17.4 per 10⁵ have been recorded respectively^[68]. UC in the Middle East appears to be of much rarer occurrence. This similarly applies for CD when comparing the Middle East to the world's highest incidence rates.

Despite the relatively rarity of disease in the Middle East homelands, some studies are able to provide temporal trends and demonstrate increasing incidence for both UC and CD. This contrasts with the stabilisation of incidence rates in many established high incidence areas in the world such as Scandinavia^[69]. This supports the hypothesis that there are risk factors specific to the western lifestyle that increase IBD incidence. The observation that IBD is more common in European-American born Jews compared to Asian-African born Jews in central Israel^[31] is consistent with this. On the other hand, such an increase may be artificial, reflecting increasing physician awareness of IBD and access to diagnostic tools. Additionally, reliability of results is reduced by lack of national population-based registries, compromising incidence and prevalence calculations.

Demographic features

There are 6 studies each^[31,32,47,50,52,58] which stratify incidence rates by sex and/or age. The female to male ratio for CD ranged from 0.74 per 10⁵ in Tel Aviv Yafo in Israel^[31] to 1.43 per 10⁵ in Southern Israel^[47], and for UC from 0.59 per 10⁵ in Trakya Turkey^[58] to 0.96 per 10⁵ in Tel Aviv Yafo in Israel^[32]. Globally, there is a slight female dominance in CD^[7,69], but overall sex differences for UC

Table 1 Summary of incidence rates for Crohn's disease and ulcerative colitis in the Middle East stratified by geographical region

Country	Region	Crohn's disease incidence rate (/10 ⁵)	Ulcerative colitis incidence rate (/10 ⁵)	Method	Study period	Ref.
Israel	Upper Galilee		2.33	Survey based study which included all cases in region	1967-1986	Niv <i>et al</i> ^[51] , 1990
	Tel Aviv Yafo	1.55		Prospective multicentre study including all hospitals and gastroenterology departments in region	1970-1980	Fireman <i>et al</i> ^[31] , 1989
	Tel Aviv Yafo		3.86	Population-based study	1970-1980	Grossman <i>et al</i> ^[32] , 1989
	Tel Aviv Yafo		3.66	Survey based multicenter study with well defined catchment area	1961-1970	Gilat <i>et al</i> ^[50] , 1974
	Tel Aviv Yafo	1.28		Survey based	1970-1976	Rozen <i>et al</i> ^[33] , 1979
	Southern Israel	4.2		Hospital and out-patient based, with complete case ascertainment	1968-1992	Odes <i>et al</i> ^[47] , 1994
	Southern Israel		2.98	Retrospective study, hospital and community clinic based	1961-1985	Odes <i>et al</i> ^[34] , 1987
	Kinneret Subdistrict		3.5	Population based study	1965-1994	Shapira <i>et al</i> ^[52] , 1998
	Kinneret Subdistrict	1.96		Hospital serving a well-defined catchment area covering all IBD cases in district	1960-1990	Shapira <i>et al</i> ^[53] , 1994
	Beer Sheva		2.87	Retrospective study, hospital based	1961-1985	Odes <i>et al</i> ^[34] , 1987
	Beer Sheva	1.1		Population-based study	1961-1980	Krawiec <i>et al</i> ^[36] , 1984
	Beer Sheva	2.1	5.4	Retrospective and prospective cohort study, hospital and community clinic based	1979-1987	Odes <i>et al</i> ^[38] , 1989
	Jerusalem		6.3	Survey based	1973-1978	Jacobsohn <i>et al</i> ^[37] , 1986
	Kibbutz settlements	5.0		Community-based survey of physicians	1987-1997	Niv <i>et al</i> ^[54] , 1999
Lebanon	Kibbutz settlements		5.04	Community-based survey of physicians	1987-1997	Niv <i>et al</i> ^[55] , 2000
	National	1.4	4.1	Population based study using a health-maintenance organisation based with large catchment area	2000-2004	Abdul-Baki <i>et al</i> ^[56] , 2007
				Questionnaire-based multicentre study	2000-2003	Tozun <i>et al</i> ^[57] , 2009
Turkey	National	2.2	4.4			
Oman	Trakya		0.77	Cross-sectional study, hospital based	1998-2001	Tezel <i>et al</i> ^[58] , 2003
	National		1.35	Prospective cohort study, tertiary referral centre based with large catchment area	1987-1994	Radhakrishnan <i>et al</i> ^[59] , 1997
Kuwait	National		2.8	Retrospective cohort study, tertiary referral centre based with large catchment area	1985-1999	Al-Shamali <i>et al</i> ^[60] , 2003
	National	0.45	2.27	Population-based study	1977-1982	Al-Nakib <i>et al</i> ^[61] , 1984

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and CD are inconsistent^[68], suggesting gender-specific risk factors may not exist. In the Middle East, incidence rates were highest in the second decade of life for CD, and third decade for UC. These peak age groups corresponded to global trend of peak age group in 20 to 29 years old range for both UC and CD^[68]. Similarly, the unimodal age distribution found by Middle Eastern studies reflects international results^[68,69], as only one third of studies demonstrate bimodal age distribution, all from westernised nations^[68]. The significance of a second peak is contentious as it may be due to missed diagnosis when the disease first presented earlier in life. On the other hand, it may indicate populations whose disease is more susceptible to environmental factors rather than genetics, and hence for countries in the Middle East where the one peak age group is relatively older, strong environmental

influences may be indicated.

Clinical features

Thirteen studies^[52-56,58-60,62,67,70-74] from the countries of Lebanon, Kuwait, Iran, Oman, Qatar and Israel characterised intestinal and/or extraintestinal involvement in CD and/or UC in Middle Eastern populations. However, classifications of the site of CD and UC intestinal involvement were not uniform in the Middle Eastern studies, nor were methods of case ascertainment and type of colonoscopy used. The most common intestinal feature reported by all the studies for CD is the terminal ileum, followed by colonic only and ileocolonic. Globally, ileocolitis is the most common^[72]. For UC, it is most commonly found as proctitis, followed by left-sided colitis and pancolitis in the Middle East. This is largely similar to the

Table 2 Summary of prevalence rates for Crohn's disease and ulcerative colitis in the Middle East stratified by geographical region

Country	Region	Crohn's disease prevalence rate (/10 ⁵)	Ulcerative colitis prevalence rate (/10 ⁵)	Method	Study period	Ref.
Israel	Upper Galilee		44.58	Survey based study which included all cases in region	1986	Niv <i>et al</i> ^[51] , 1990
	Tel Aviv Yafo	13.28		Prospective multicentre study including all hospitals and gastroenterology departments in region	1970-1980	Fireman <i>et al</i> ^[31] , 1989
	Tel Aviv Yafo		55.16	Population-based study	1980	Grossman <i>et al</i> ^[32] , 1989
	Tel Aviv Yafo		37.4	Survey based multicenter study with well defined catchment area	1970	Gilat <i>et al</i> ^[18] , 1974
	Tel Aviv Yafo	12.31		Survey-based	1976	Rozen <i>et al</i> ^[33] , 1979
	Southern Israel	50.60		Hospital and out-patient based, with complete case ascertainment	1992	Odes <i>et al</i> ^[47] , 1994
	Southern Israel (Arab population)	3.20	9.8	Prospective cohort study, hospital and community clinic based	1990	Odes <i>et al</i> ^[62] , 1991
	Kinneret Subdistrict	20.24		Hospital based serving a well-defined catchment area covering all IBD cases in district	1960-1990	Shapira <i>et al</i> ^[53] , 1994
	Beer Sheva		70.6	Retrospective study, hospital based	1985	Odes <i>et al</i> ^[34] , 1987
	Beer Sheva	14		Population-based study	1980	Krawiec <i>et al</i> ^[36] , 1984
	Beer Sheva	30	89.0	Retrospective and prospective cohort study, hospital and community clinic based	1987	Odes <i>et al</i> ^[38] , 1989
	Kibbutz settlements	65.1		Community-based survey of physicians	1987-1997	Niv <i>et al</i> ^[54] , 1999
	Kibbutz settlements		144.1	Community-based survey of physicians	1987-1997	Niv <i>et al</i> ^[55] , 2000
	Kibbutz settlements		121.08	Community-based survey of physicians	1987	Niv <i>et al</i> ^[63] , 1991
	Kibbutz settlements		168.3	Survey based, complete case ascertainment and large catchment area	1987-2007	Birkenfeld <i>et al</i> ^[66] , 2009
	Kibbutz settlements	67.9		Survey based, complete case ascertainment and large catchment area	1987-2007	Zvidi <i>et al</i> ^[64] , 2009
Lebanon	National	53.1	106.2	Population based study using a health-maintenance organisation based with large catchment area	2007	Abdul-Baki <i>et al</i> ^[56] , 2007
Turkey	Trakya		4.9	Cross-sectional study, hospital based	2002	Tezel <i>et al</i> ^[58] , 2003
Kuwait	National		41.7	Retrospective cohort study, tertiary referral centre based with large catchment area	1985-1999	Al-Shamali <i>et al</i> ^[60] , 2003

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extent of UC in other low incidence countries. The most common extraintestinal involvement was rheumatological conditions, a finding reflected worldwide^[73].

Risk factors

Ten studies investigated the risk factor profiles in UC and/or CD populations studied^[54-59,64,67,71]. However, only one study - an Iranian study with 258 cases^[67] - included controls, highlighting an area that would benefit from more case control studies. The most common risk factor studied was smoking, and generally studies showed smoking as less prevalent in UC subjects, and the opposite for CD. This is consistent with global findings. Results for other risk factors are largely inconsistent between Middle Eastern studies.

Racial/ethnic differences

Due to significant differences in IBD incidences within different ethnic groups in Israel, such that IBD incidence was highest among American and European born Jews

than those born in Israel, Asia and Africa^[56], it is worthwhile for future studies to distinguish IBD characteristics according to ethnicity. Interestingly, it is observed that the prevalence gap between different ethnic groups is disappearing in the Kibbutz settlements, Tel Aviv Yafo and the South and North of Israel^[66]. Studies also show that the risks of developing UC for European and American born Jews and for Jews born in Israel are becoming very similar^[34]. It can be inferred that as these countries become increasingly westernised, IBD becomes more prevalent. Further studies are necessary to identify the environmental components, ideally done through investigating generational changes when there is migration from developing to developed countries.

EPIDEMIOLOGY OF IBD IN AUSTRALIA

Incidence and prevalence

Wilson *et al*^[74] conducted the first and only prospective population based study to determine Australia's IBD inci-

dence over a period of 1 year using the capture-recapture method with good sample size. The crude annual incidence rate for overall IBD, UC and CD were 29.3 per 10⁵, 17.4 per 10⁵ and 11.2 per 10⁵ respectively. This result in the overall IBD incidence rate of 29.6 per 10⁵ after age-standardised to the World Health Organisation world standard population^[74]. When compared to global IBD incidence, the most recent systematic review of regional epidemiology demonstrates that Australia has higher UC rates than Europe (12.7 per 10⁵), Asia and Middle East combined (6.3 per 10⁵), and lower when compared to North America. For CD, compared to Europe (12.7 per 10⁵) and North America (20.2 per 10⁵), Australia's rates are less, while higher than Asia and the Middle East (5.0 per 10⁵)^[68]. This study is critical in confirming Australia's high IBD incidence which was not previously established; additionally it supports the assumption that Australia shares similar genetic and environmental risk factors with other westernised nations^[74].

An earlier smaller study was conducted in the Hunter Valley region of New South Wales on CD patients between 1967 and 1991. A mean incidence of 1.38 per 10⁵ with a 3-fold increase in incidence rates between the first and second 10 years was found. The prevalence of these patients was 34 per 10⁵. There was a female dominance (1.4:1) and the most common intestinal involvement site was ileocolonic, followed by colonic only, and jejuna^[75]. The study's inclusion of only surgically managed CD means there is an underestimation of the total CD incidence in the area.

The short time period of the study conducted by Wilson *et al*^[74] is the main limiting factor. Hence in actuality IBD incidence in Australia could be higher. Additionally, further population-based studies conducted over longer periods should stratify results by ethnic subgroups to better represent Australia's IBD epidemiology considering the multiculturalism of this country.

Clinical features and environmental risk factors

Inflammatory bowel disease presentation in Australia is comparable to many Westernised nations. The bimodal age distribution of IBD in Australia, with 20-24 year old age being the peak age group^[74], reflects the findings of some Westernised nations^[68]. Phenotypically, for CD terminal ileum involvement is most common (49%), followed by colonic only disease (27%) and ileocolonic (24%). For UC, proctosigmoiditis or left sided colitis was most common (48%), followed by isolated proctitis (35%) and pancolitis (17%)^[74]. Thus far, there is a lack of epidemiological studies focused on the adult population on environmental risk factors of IBD. A retrospective study on children in the state of Victoria found high prevalence of urban dwelling in cases.

Migrant populations

Multicultural diversity distinguishes Australia. With net overseas migration comprising 53% of Australia's annual population growth^[76] it is evident that several generations

of migrants populate the country, causing it to be a prime setting to study environmental risk factors of IBD when tracing generational changes. The 2006 census data indicates 193633 people born in the Middle East who were resident in Australia. This accounted for 4.4% of the overseas-born population. Approximately 40% were born in Lebanon, 16.8% were born in Iraq, 15.7% in Turkey, 11.6% in Iran, 4.0% in Israel, 3.6% in Syria and 1.9% in Jordan^[77]. Most of the Middle-Eastern - born population reside in Sydney (58.1%). Arrival peaks occurred in 1977 and 1987 coinciding with the Lebanese Civil War. The union of individuals with a common ancestor known as consanguinity is found in the Middle East with a prevalence of 26% in 1988^[78]. As IBD has a genetic component it may therefore be more likely to manifest in this migrant group given suitable environmental condition. This migration of Middle Eastern populations to Australia therefore allows for the study of the development of IBD and allows for comparison of first- and second-generation migrants.

CONCLUSION

Migrant studies exploring generational IBD changes and risk factor profiles contribute towards greater understanding of IBD environmental influences due to the advantages they confer. Modern migration patterns offer unique opportunities for these epidemiological studies. Identifying and studying suitable migrant groups, such as Middle Eastern migrants in Australia, is likely to increase our understanding of the environmental triggers of IBD which thus far remain ambiguous entities.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

What is left when anti-tumour necrosis factor therapy in inflammatory bowel diseases fails?

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Abstract

The inflammatory bowel diseases (IBDs) are chronic incurable conditions that primarily present in young patients. Being incurable, the IBDs may be part of the patient's life for many years and these conditions require therapies that will be effective over the long-term. Surgery in Crohn's disease does not cure the disease with endoscopic recurrent in up to 70% of patients 1 year post resection. This means that, the patient will require many years of medications and the goal of the treating physician is to induce and maintain long-term remission without side effects. The development of the anti-tumour necrosis factor alpha (TNF α) agents has been a magnificent clinical advance in IBD, but they are not always effective, with loss of response overtime and, at times, discontinuation is required secondary to side effects. So what options are available if of the anti-TNF α agents can no longer be used? This review aims to provide other options for the physician, to remind them of the older established medications like azathioprine/6-mercaptopurine and methotrexate, the less established medications like mycophenolate mofetil and tacrolimus as well as newer therapeutic op-

tions like the anti-integrins, which block the trafficking of leukocytes into the intestinal mucosa. The location of the intestinal inflammation must also be considered, as topical therapeutic agents may also be worthwhile to consider in the long-term management of the more challenging IBD patient. The more options that are available the more likely the patient will be able to have tailored therapy to treat their disease and a better long-term outcome.

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Key words: Inflammatory bowel disease; Immunosuppression; Anti-tumour necrosis factor agents; Anti-integrin; Long-term outcomes

Core tip: Overall the physician must keep an open mind when treating inflammatory bowel disease. These patients have a long-term incurable condition than can significantly impact on all aspects of their life. Surgery does not cure the disease and thus medications may be required for many decades in order to give the patients a decent quality of life. Both the patient and the physician, therefore, need to remember the "oldies but goodies" but also keep the door open to new innovations and novel therapies.

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INTRODUCTION

The chronic inflammatory bowel diseases, Crohn's disease (CD) and ulcerative colitis (UC), are a huge challenge for the treating physician as these are life-long incurable

conditions that frequently present in the 2nd or 3rd decade of life, a stage in the patient's life where education, social integration and personal identity are key aspects being developed. There is no doubt that the anti-tumour necrosis factor alpha (TNF α) medications are efficacious in the management of both conditions^[1-8] but they are, however, not a panacea as they are not effective in all patients and even in those in whom a remission is achieved, the effect may be lost over time.

The efficacy of maintenance therapy in CD with the anti-TNF α medication, certolizumab pegol, has been investigated out to 18 mo. This observed that slightly more than 60% of the original 60% of patients who responded to induction therapy continued to respond, which is encouraging^[9,10]. This suggests, however, that by 18 mo only 40% of patients are still getting benefit from this medication. This is similar to the findings for adalimumab where 24% of all patients in the CHARM study remained in response at week 26^[3] and after 2 years of adalimumab therapy, between 37% and 50% of these patients were in clinical remission^[11]. Published data for infliximab out to 54 wk would also appear to be similar^[12]. Additional long-term data, out to 4.5 years, suggest that although efficacy for certolizumab in CD is still present^[13], the number of patients continuing to benefit falls with time. The problem for both the patient and physician is that the IBDs are life-long conditions and arguably the best medication options for these patients have only a limited subset of patients in whom they will be of long-term benefit.

In addition to a loss of effect over time, like all medications, there are potential side effects to the use of the anti-TNF α agents. As TNF is involved in the immune-mediated response to infection it is not unexpected that anti-TNF medications are associated with an increased risk of serious and opportunistic infections^[14,15], including tuberculosis^[16], *Pneumocystis jirovecii* pneumonia^[17], and various viral, fungal and bacterial infections^[18]. Dermatological side effects are also possible with new onset cutaneous eruptions observed in 20% of CD patients treated with infliximab^[19], and immune-mediated cutaneous reactions seen in 11% of patients^[20]. This risk is also present with the fully humanised antibody, adalimumab^[21]. In addition, the potential risks of medication-induced skin cancers and lymphomas need to be considered^[22].

Thus these long-term chronic inflammatory diseases, which may be part of a patient's life anywhere from 10 and 70 years, require medications that will be effective over the long-term with minimal side effects. The development of the anti-TNF α agents has been a magnificent clinical advance in this management of these conditions, but what options are available if they lose effect or side effects necessitate cessation of the therapy?

OLD BECOMES NEW AGAIN

Azathioprine and 6-mercaptopurine

An oldie but a goodie. We must never forget about the older medications that have stood the test of time as they

are still frequently used and with more innovative thinking may be able to either enhance the effects of the anti-TNF α agents, or be a backstop if, or when, they are no longer of benefit. Through their effects on the synthesis of nucleic acids, the thiopurines reduce intracellular purine metabolism, induce T lymphocyte apoptosis, cause a reduction in the number of circulating B and T lymphocytes^[23], decrease immunoglobulin synthesis^[24] and reduce the production of interleukin (IL)-2^[25] with the desired effect of reducing inflammation.

In many IBD centres, the measurement of thiopurine methyltransferase (TPMT) activity is frequently undertaken as this is the primary determinant of azathioprine (AZA)/6-mercaptopurine (6-MP) metabolism. For patients with moderate enzymic activity (5-12 U/mL), they are likely to achieve 6-thioguanine nucleotides (6-TGN) levels at standard drug dosing (AZA 1.5 mg/kg/6-MP 1.0 mg/kg), while patients with high enzyme activity (usually > 12 U/mL) may require higher doses than normal. TPMT activity, however, does fluctuate, and TPMT enzymic activity can be induced by AZA/6-MP therapy, while 5-aminosalicylates may cause a mild, but reversible, inhibition of TPMT activity.

The measurement of 6-TGN and 6-methylmercaptopurine (6-MMP) levels are now also frequently undertaken as these levels can correlate with the therapeutic response. A 6-TGN level of between 230-400 pmol/8 \times 10⁸ RBC has been associated with clinical response, although this data needs to be re-examined in a larger patient cohort. Of note is that 6-TGN levels > 400 pmol/8 \times 10⁸ RBC are often associated with myelosuppression, while 6-MMP levels of > 5700 pmol/8 \times 10⁸ RBC can be a cause of hepatotoxicity and other AZA/6-MP-induced side effects^[26-29].

Of particular note is that the 6-TGN and 6-MMP levels can be used to determine a patient's compliance and may indicate high TPMT activity with shunting of thiopurine metabolism towards the 6-MMP metabolite and away from 6-TGN. If shunting is observed with high 6-MMP and low 6-TGN levels, the addition of allopurinol (100 mg/d) appears to increase the activity of hypoxanthine-guanine phosphoribosyltransferase, which is the first step in the metabolism of the thiopurines to 6-TGN, resulting in increased 6-TGN levels^[30-32]. If allopurinol is used then the AZA/6-MP dose must be markedly reduced, generally the author would reduce it to 25% of the original dose until rechecking of the metabolite profile^[33].

If there is loss of response to anti-TNF α therapy then the combination of AZA/6-MP with the anti-TNF α agent could also be of benefit. It is now accepted that the combination of the thiopurines with the anti-TNF α s is more effective for the induction and maintenance of steroid-free remission, and mucosal healing in CD than with the use of either drug alone for up to 1 year in patients who are naïve to both agents^[34,35]. The evidence for reclaiming a response to anti-TNF α therapy once lost is not clear, but it is at least a viable option for consideration. The evidence of the combined use of

these agent in UC, however, is not as strong as in CD, but as there is a role for AZA/6-MP in mucosal healing, and protection against the development of colorectal cancer the combination of the two agents would again seem to be reasonable to consider^[36].

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is a immunosuppressing agent with similar anti-metabolite and pharmacodynamic properties to the thiopurines, which has primarily been used for preventing the rejection of solid organ transplantants. Its role as an immunosuppressant in the management of IBD has to date been fairly limited with several open labelled studies^[37-39] and only a few randomized trials that have been limited by low patient numbers^[40-42]. Consideration of its use in the management of difficult IBD cases, however, should not be ignored.

Most early studies investigating the use of MMF were undertaken in CD patients who had failed, or were intolerant to, AZA, and these demonstrated good efficacy^[40,42,43]. Unfortunately, these findings were not always reproduced by later studies. These later studies suggested that there was both a low initial response rate as well as a high relapse rate. It was also noted that there was frequently a high medication discontinuation rate secondary to side effects^[37,38,41,44,45]. Additional studies comparing the efficacy of AZA to MMF, however, observed that MMF could be more effective in AZA intolerant, rather than refractory patients, while being non-inferior to AZA in the management of UC, for the induction and maintenance of remission at 6 mo^[40,46,47]. A longer-term study in a cohort of AZA resistant/intolerant patients, however, observed that although MMF was initially effective, the relapse rates were high, with the suggestion that MMF may be potentially effective but not a long-term solution^[48]. There has also been suggested that the MMF dose needs to be increased over time in order to maintain an effect. This has not been the experience of the author as our data demonstrate that MMF was efficacious and well tolerated in treating refractory IBD who are intolerant to AZA/6-MP without the problems of an early disease flare, or the need for dose escalation over time^[39].

As many of the studies suggest that MMF is potentially as effective as conventional immunosuppressants when these medications fail, or cannot be used due to hypersensitivity reactions including pancreatitis, then it is a potential alternative that is worthwhile for consideration^[46,47]. Further evaluation of its role needs to be undertaken in larger randomized, double-blind studies comparing it to conventional immunosuppressants, however, such studies are expensive and not easy to undertake.

Methotrexate

Methotrexate (MTX) exerts its activity at the DNA level. It inhibits the conversion of dihydrofolic acid to folinic acid, its active metabolite, through the competitive inhibition of dihydrofolate reductase. As folinic acid is required for purine and pyrimidine metabolism and

amino acid synthesis, MTX alters their incorporation in the DNA and reduces cellular proliferation, increases T cell apoptosis and endogenous adenosine with alteration of the expression of cellular adhesion molecules and the production of proinflammatory cytokines. The resultant effect is a reduction on systemic inflammation.

Unfortunately, there have been only limited studies investigating MTX in IBD. The largest trial investigated the use of 25 mg/wk intramuscular (*im*), or placebo, in combination with 20 mg/d prednisolone^[49]. At 16 wk, significantly more patients receiving MTX were in remission off steroids compared to placebo ($P = 0.025$), however, adverse events were significantly more common with MTX. Two other small trials examining oral MTX 15 mg/wk^[50] for 3 mo compared to placebo and oral MTX 12.5 mg/wk in combination with 50 mg/d 6-MP or placebo for 9 mo^[51] demonstrated no significant differences between the groups. The second study, however, used suboptimal doses of the immunomodulators, did not have well defined steroid reduction protocols and included patients with known thiopurine-resistant disease.

The use of MTX has been further examined in two open-label studies in CD, the first compared 25 mg/wk MTX intravenously for 3 mo followed by 25 mg/wk MTX orally, with 2 mg/kg per day oral AZA^[52] for 6 mo. At 3 and 6 mo there was no difference between the percentage of patients in remission between the MTX and AZA groups, but there were significantly more adverse events with MTX. The second study examined patients naïve to immunomodulator therapy^[53] and compared MTX 15 mg/wk orally with 6-MP 1.5 mg/kg per day and 5-ASA 3 g/d for 30 wk. Remission was achieved in 80% of patients on MTX and 94% on 6-MP, but this was not statistically different.

The combination of MTX and the anti-TNF medications has been suggested to be beneficial in paediatric patients with one retrospective analysis^[54], and the findings are similar to those seen with the combination of thiopurines and an anti-TNF agent in CD. Expert opinion is also that the combination MTX and an anti-TNF agent can be of benefit in the adult IBD population^[55], particularly when the anti-TNF therapy is used episodically. In addition, although the data on the effect that MTX has on mucosal healing is very limited with only a single case series of in CD patients, it does suggest that MTX does have the potential for mucosal healing^[56].

Despite the limited number of studies of MTX in the induction and maintenance of remission in CD the conclusion of the Cochran review was that MTX was useful in steroid dependent CD and should be commenced at 25 mg/wk *im* of subcutaneous (SC) and continued for 16 wk^[57-59]. Maintenance of remission could then be continued with MTX at 15 mg/wk *im* or SC but with no evidence to recommend the use of oral MTX^[60]. The evidence for MTX in UC is even more limited with a single retrospective case series suggesting some benefit, and only a single prospective randomised trial^[51]. The efficacy in UC thus appears to be primarily based on anecdotal

experience alone and this is reflected in the Cochran review which stated that there was no evidence for MTX treatment in UC^[61-63].

Tacrolimus

Tacrolimus is a macrolide immunosuppressant that is frequently used to prevent the rejection of renal and hepatic allografts. It has the ability to inhibit T cell activation through the formation of an intracellular complex with immunophilins^[64] and these bind to, and inhibit, calcineurin, an enzyme involved in the regulation of transcription factors. Tacrolimus thus does not exert its effect through the inhibition of DNA synthesis but instead inhibits T lymphocyte proliferation through the inhibition of proliferative cytokine production like IL-2^[65].

Its role in the management of IBD has been investigated in a number of studies, but unfortunately the majority of these are small, retrospective and uncontrolled^[66-78]. In general there would appear to be some efficacy with remission rates ranging from 7%-69% in patients with luminal CD^[79] and 9%-74%^[70,80] in those with UC. The durability of the clinical response, however, is something that may not be optimal^[72] with significant variability in the findings and this, in combination with the potential adverse effects of headache, tremor, paraesthesia, insomnia, gastrointestinal upset, arthralgia and particularly nephrotoxicity^[73,81], makes the use of tacrolimus in IBD somewhat controversial and its use is not wide spread.

Tacrolimus was initially used in the management of perianal CD where for 10 wk patients received tacrolimus 0.1 mg/kg per day or placebo^[82]. There was a significant reduction in fistulae drainage in the tacrolimus-treated group of 43% compared to the placebo group of 8% ($P = 0.01$), however there was no difference in the remission rates between the groups.

The rest of the data for luminal CD comes primarily from retrospective case series that focus on patients who have failed, or are intolerant, to AZA/6-MP. There are also studies that have included patients failing anti-TNF α therapy, but the proportion of these patients is generally low and are in the range from 13% to 47%^[67,70,83]. These studies, however, do suggest some efficacy in both the induction and maintenance of remission^[68,70,72] with most patients commencing on 0.1 mg/kg tacrolimus twice a day with the aim to get the tacrolimus trough level within the therapeutic range of 5-20 ng/L. Better response and remission rates would appear to be associated trough levels of > 10 ng/L, and these can reach a response rate of between 68% to 83%^[73] and a remission rate of 64% in CD patients^[72].

In UC patients hospitalised with moderate/severe steroid refractory disease^[81], not on AZA/6-MP, the use of tacrolimus after two weeks was associated with a clinical improvement that was statistically significant, and dose dependent, suggesting that high serum concentrations (10-15 ng/L) are more efficacious than low concentrations (5-10 ng/L) or placebo. Tacrolimus was also noted

to achieved mucosal healing in 78.9% (15/19) of patients with the high trough levels compared to 44.4% (8/18) of patients with low trough levels and placebo 12.5% (2/16)^[81]. The most recent Cochran review^[84] also concluded that tacrolimus was effective in inducing a clinical improvement in a dose-dependent manner in treatment-resistant UC with the number needed to treat being 3.

More recently the long-term efficacy of tacrolimus in both CD and UC patients who had failed standard immunosuppressive and anti-TNF α therapy was assessed in a retrospective study with a trough level targeted between 8-12 ng/mL^[85]. Clinical response, remission and surgery were then assessed at 30-d, 90-d and 1-year. This paper identified that 65.7% of patients had a clinical response at 30 d, 60% at 90 d and 31.4% at 1 year while 40% of patients were in remission at 30 d, 37.1% at 90 d and 22.9% at 1 year. The risk of surgery was significantly reduced in patients who achieved and maintain a clinical response at 90-d ($P = 0.004$). The risk of surgery at 1 year was still very high at 40% and almost 60% by 2 years, but the figures were similar to the 50% three year colectomy rate observed in steroid refractory UC patients treated with infliximab as rescue therapy^[86]. The findings thus suggest that tacrolimus could induce both a clinical response and clinical remission in medically refractory IBD patients with long-term benefits.

TOPICAL THERAPIES

In all conditions the disease location, severity, patient preferences and allergies need to be considered when prescribing any treatment. Topical tacrolimus has been effective in the treatment of both the perioral and perineal inflammation present in paediatric CD, with resolution of symptoms in up to 75% of patient^[87]. More aggressive or novel topical therapy may also be of benefit in distal UC^[88,89]. Distal ulcerative colitis (DC), also known as left-sided colitis or E2 disease under the Montreal classification^[90], is disease confined to the colon distal to the splenic flexure, while proctitis, or E1 disease (Montreal classification), is disease localized to the rectum. These occur in over 50% of UC patients and, although these result in distressing symptoms, including increased stool frequency, tenesmus, urgency and bleeding, they can often be managed within the community. Resistant disease, however, can be extremely difficult to manage and when there is failure of disease control with routine topical 5-aminosalicylic acid (5-ASA) and steroid therapy, oral agents including the 5ASAs, AZA/6-MP, steroids or an anti-TNF α medication may be use. Unfortunately they do not always help and clinical remission with anti-TNF α therapy only occurs in at most a third of patients^[7,91].

It is in these patients that the investigation of other agents is required. To date there have been numerous topical agents proposed for left-sided disease and these have been investigated primarily by open-labelled studies including tacrolimus suppositories^[92,93], as well as tacrolimus^[93], ecabet sodium^[94,95], acetarsol^[96] and thromboxane

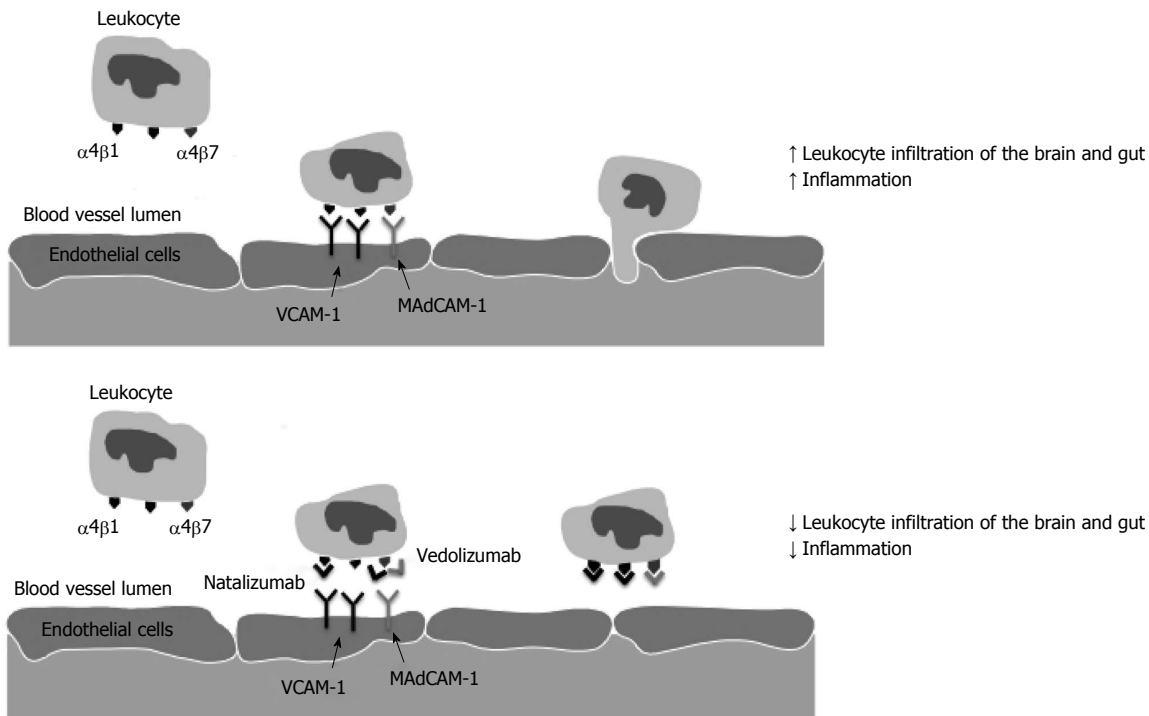


Figure 1 Modification of Leukocyte trafficking by the anti-integrins. VCAM-1: Vascular cell adhesion molecule-1; MAdCAM-1: Mucosal addressin cell adhesion molecule 1.

enemas^[97]. Unfortunately none of these have undergone blinded randomised studies as yet, although tacrolimus suppositories are currently being investigated in a double-blind placebo-controlled trial. There are, fortunately, several other agents that have undergone randomised studies, and these include butyrate^[98-100], cyclosporine^[101] and nicotine enemas^[102], however, none have demonstrated better efficacy than placebo in left-sided disease or proctitis. In addition, despite impressive evidence for epidermal growth factor enemas in one small randomized study, the finding has never been reexamined or reproduced^[103]. It does appear, however, that the mucosal medication concentration and/or contact time may be important for the topical agents to work^[104]. This suggests that enemas are not the best method of administration for patients with proctitis. Further investigation is still required, however, before any of these agents can be considered as routine in the management of DC or proctitis, but the need is great and hopefully further work will be undertaken.

MODIFYING T CELL TRAFFICKING

As inflammation in IBD is thought to result from inappropriate activation of the mucosal immune system by intestinal luminal antigens in genetically susceptible individuals^[20,105], the trafficking of leukocytes into the intestinal mucosa would appear to be central to the induction, and maintenance, of the intestinal inflammation in IBD. Trafficking of leukocytes is mediated *via* the recognition of specific adhesion molecules, or integrins that are heterodimeric glycoproteins located on the cellular membrane^[106]. These transmembrane receptors consist of an

α - and β -subunit with at least 24 different combinations already identified allowing for a wide range of receptor specificity^[107]. The α subunit determines the specificity of the interaction between the leukocyte and the endothelial cell and the $\alpha 4$ integrin is widely expressed in both the intestine and brain, and is able to form two different heterodimers with either the $\beta 1$ or $\beta 7$ -subunit (Figure 1)^[107].

The $\alpha 4\beta 1$ integrin is primarily expressed on lymphocytes and monocytes^[108], and binds with vascular cell adhesion molecule-1 that is located on vascular endothelial cells allowing cellular migration into the tissue matrix of the brain¹⁰⁹. The $\alpha 4\beta 7$ integrin demonstrates some overlapping specificity with the $\alpha 4\beta 1$, but also recognises mucosal addressin cell adhesion molecule-1 (MAdCAM-1) that is important in trafficking of lymphocytes into the gut^[109,110]. Of particular note is that MAdCAM-1 expression levels are known to be upregulated in association with chronic inflammation in both the small and large intestine of patients with both CD and UC^[111,112] and that the $\alpha 4\beta 7$ heterodimer is highly expressed on memory T cells within the intestine^[113].

In addition to the integrins there are other proteins that are found on the cell surface of circulating lymphocytes. One of these is chemokine receptor 9 (CCR9) and it is the only known ligand for CCL25, which is expressed by gastrointestinal tract epithelial cells^[114]. When CCR9 is expressed on circulating lymphocytes these cells are able to traffic to the intestine^[115,116] and thus CCR9 has been implicated in the development and maintenance of the inflammation observed in IBD^[117]. Thus modifying the trafficking of these cells may also impact on the development and progression of IBD inflammation.

Natalizumab

The first of the medications to test the concept of altering leukocyte trafficking was Natalizumab (Tysabri, Elan Pharmaceuticals and Biogen Idec), which is a humanised anti-integrin to IgG₄ monoclonal antibody that bound to, and inhibited that binding of the $\alpha 4$ integrin to its target proteins in the brain and the gut and it was shown to be effective in the treatment of multiple sclerosis^[118,119]. In the 12-wk induction trial in moderate to severe CD patients, patients were randomly assigned in a 4:1 ratio to receive Natalizumab or placebo with the primary endpoint at week 10 and this was defined as a clinical response with a drop in the CD activity index (CAI) of ≥ 70 points^[120]. Although the primary end point was not met ($P = 0.05$) post hoc analysis identified that if a CDAI drop of > 100 was used or if patients were stratified for an elevated C-reactive protein at baseline, significance was detected between the groups. In the maintenance study, Natalizumab, however, demonstrated an ability to maintain a clinical response ($P < 0.001$) and remission ($P = 0.003$) compared to placebo. Unfortunately the emergence of the life threatening side effect, progressive multifocal leukoencephalopathy (PML), was associated with the use of Natalizumab and due to this the FDA added the criteria that Natalizumab must not be used in combination with immunosuppressants or inhibitors of TNF- α , and the use of Natalizumab for the management of CD has never been approved in many countries.

Vedolizumab

Natalizumab demonstrated that altering lymphocyte trafficking could effect site-specific inflammation^[121,122]. But as this anti- $\alpha 4$ monoclonal antibody targeted both the $\alpha 4\beta 1$ and $\alpha 4\beta 7$, and was associated with an increased risk of PML, the potential of targeting the $\beta 7$ subunit, or both the $\alpha 4$ and $\beta 7$ subunits was considered. This would improve antibody specificity by only affecting those leukocytes homing to the intestine, and potentially would have less systemic side effects.

The humanised monoclonal antibody Vedolizumab (Millennium: The Takeda Oncology Company, Cambridge, MA, United States) was developed as a highly selective adhesion molecule antagonist that blocked the interaction between the $\alpha 4\beta 7$ integrin and its ligand thus preventing lymphocyte migration into the gut^[123]. Recently the phase III induction and maintenance studies for both CD and UC have been presented with very encouraging results. In UC there were response rates at 6 wk of 47.1% in the treatment arm [300 mg intravenously (*iv*) at weeks 0 and 2] compared to 25.5% of patients receiving placebo ($P < 0.001$), while maintenance therapy with *iv* Vedolizumab at either 4 or 8 weekly was compared to placebo and the percentage of patients who were in clinical remission at week 52 was 41.8, 44.8 and 15.9 respectively ($P < 0.001$ both treatment arms to placebo).

The use of Vedolizumab in CD has also been encouraging. The induction phase was the same as for the UC study and at week 6, 31.4% of patients on Vedolizumab

had a clinical response compared to 25.7% of patients on placebo ($P = 0.23$) but 14.5% of patients on active treatment were in remission compared to 6.8% receiving placebo ($P = 0.02$). At week 52, however, the percentage of patients who were in clinical remission was 39.0% (4 weekly infusion), 36.4% (8 weekly infusion) compared to 21.6% receiving placebo ($P < 0.0001$ and $P = 0.004$ respectively). There was noted to be a higher risk of adverse events for patients receiving Vedolizumab, but there were no cases of PML, compared to those getting placebo suggesting that further experience and data collection will be required.

Vercirnon

Vercirnon (CCX282-B) is a selective antagonist of CCR9 with the advantage of being orally active^[124] that was initially synthesised by ChemoCentryx Inc, but was subsequently investigated by GlaxoSmithKline where it has just completed the pivotal induction study and was to continue investigation in the SHEID studies for the management of CD. The preclinical studies demonstrated that this molecule inhibits the CCL25-induced chemotaxis^[125] and in animal models of colitis, was shown to reduce the severity of intestinal inflammation in the TNF^{ΔARE} murine model of colitis^[125].

In the two Phase II/III parallel studies, Vercirnon was examined in moderate to severe active CD. The percentage of patients achieved a clinical response (CAI decrease ≥ 70 points from baseline), or remission (CAI < 150), at 12 wk CD was 61% and 29.9% compared to those getting a placebo of 47.2% ($P = 0.039$) 27.1% (not significant) respectively. The percentage of patients in remission at 52 wk was 47% in the treatment arm and 31% with placebo ($P = 0.012$) suggesting that there was potentially some efficacy of the medication.

Further studies, however, have been unimpressive with the SHIELD-1 study undertaken by GSK determining that in adult patients with moderately-to-severely active CD, Vercirnon did not achieve the primary endpoint of improvement in clinical response nor the key secondary endpoint of clinical remission. Of note was that although the rates of serious adverse events, and withdrawals due to adverse events, were similar among the groups, there was a trend for a dose-dependent increase in overall adverse event rates with Vercirnon. Consequently, GSK has ceased all clinical trials into the use of Vercirnon in management of CD until there have been further analysis of the SHIELD-1 findings.

CONCLUSION

There is no doubt that the anti-TNF α medications have been a great addition to the treatment options for both UC and CD with many promoting a "top down" therapeutic approach that commences with an anti-TNF α medication in the management of CD, or a rapid "step up" approach when this is not feasible. These medications, however, do not always induce remission and loss

of response over time, or the development of side effects, may also limit their long-term efficacy.

In all cases the location and severity of the intestinal inflammation will determine which medications are required and the best mode of administration. For the left sided and distal colidities, and perianal CD, topical agents may be the best choice. Particular thought should be put into this by the physician as these often have less systemic side effects than other agents and can be every effective. Unfortunately further investigation is required before many of these will be part of routine management.

The thiopurines have demonstrated long-term efficacy and by measurement of their metabolites and modification of their activity of hypoxanthine-guanine phosphoribosyltransferase with allopurinol, their efficacy may be increased. Use in combination with the anti-TNF α medications is also of benefit and should be considered in all patients in order to prolong and improve the long-term outcomes with these medications. Methotrexate may also be able to be used in a similar manner to the thiopurines and improved patients outcomes. Less recognized are MMF and tacrolimus as medications for use in IBD but these should also be considered when conventional therapies fail or patient intolerances limit the use of conventional therapies.

There are now newer therapies that have been developed to target leukocyte trafficking to the intestine and these have, fortunately, demonstrated clinical efficacy. The most recent is Vedolizumab, which blocks the $\alpha 4\beta 7$ integrin and it achieved demonstrated impressive efficacy for the induction and maintenance of remission in UC and also has a long-term effect on the maintenance of remission in CD. It would thus be expected that very soon this medication will be under consideration by the various regulatory authorities around the world for use in the IBDs thus allow a further therapeutic option.

Overall the physician must keep an open mind when treating IBD. These patients have a long-term incurable condition than can significantly impact on all aspects of their life. Surgery does not cure the disease and thus medications may be required for many decades in order to give the patients a decent quality of life. Both the patient and the physician, therefore, need to remember the “oldies but goodies” but also keep the door open to new innovations and novel therapies.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease***Campylobacter concisus* and inflammatory bowel disease**

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Abstract

Investigation of the possible role of *Campylobacter concisus* (*C. concisus*) in inflammatory bowel disease (IBD) is an emerging research area. Despite the association found between *C. concisus* and IBD, it has been difficult to explain how *C. concisus*, a bacterium that is commonly present in the human oral cavity, may contribute to the development of enteric diseases. The evidence presented in this review shows that some *C. concisus* strains in the oral cavity acquired zonula occludens toxin (*zot*) gene from a virus (prophage) and that *C. concisus* *Zot* shares conserved motifs with both *Vibrio cholerae* *Zot* receptor binding domain and human

zonulin receptor binding domain. Both *Vibrio cholerae* *Zot* and human zonulin are known to increase intestinal permeability by affecting the tight junctions. Increased intestinal permeability is a feature of IBD. Based on these data, we propose that a primary barrier function defect caused by *C. concisus* *Zot* is a mechanism by which *zot*-positive *C. concisus* strains may trigger the onset and relapse of IBD.

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Key words: *Campylobacter concisus*; Inflammatory bowel disease; Zonula occludens toxin; Tight junctions; Intestinal permeability

Core tip: *Campylobacter concisus* (*C. concisus*) is an oral bacterium that was previously shown to be associated with inflammatory bowel disease (IBD). Evidence presented in this review shows that some strains of *C. concisus* acquired zonula occludens toxin (*zot*) gene from a virus (prophage), suggesting that a primary barrier function defect caused by *C. concisus* *Zot* is a mechanism by which *zot*-positive *C. concisus* strains may trigger the onset and relapse of IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract^[1]. The two major clinical forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD is not fully understood. Multiple contributors including genetic

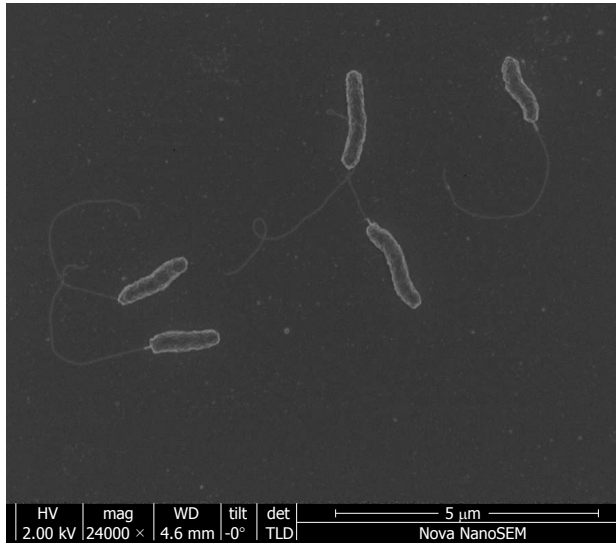


Figure 1 Electron microscopic image of *Campylobacter concisus*.

factors, environmental factors and intestinal microbiota have been suggested to play a role in the development of IBD^[1]. The pathogenesis of IBD is thought to result from a dysregulated response of the intestinal mucosal immune system to luminal commensal microbes^[1].

The highest incidence of IBD, both CD and UC, is in young adults^[2]. This implies that in most of the patients with IBD, the intestinal mucosal immune system has maintained a non-hostile relationship with the intestinal commensal microbes for decades prior to the onset of the disease. Given this, the uncontrolled attack of the intestinal mucosal immune system to luminal commensal bacteria would have been initiated by a trigger. Such a trigger may not necessarily be a dominant intestinal bacterial species or a long-term intestinal resident microbe. Evidence presented in this review suggests that zonula occludens toxin gene (*zot*)-positive *Campylobacter concisus* (*C. concisus*) strains, a bacterium colonizing the human oral cavity, may be a trigger of IBD.

C. concisus

C. concisus is a Gram negative bacterium that requires microaerobic or anaerobic conditions enriched with H₂ for growth^[3]. Cells of *C. concisus* are curved, with a size of (0.5 - 1) × (2 - 6) μm. *C. concisus* is motile, driven by a single polarized flagellum (Figure 1).

HUMANS ARE THE NATURAL HOST OF *C. CONCISUS* AND THE ORAL CAVITY IS THE PRIMARY COLONIZATION SITE

Tanner *et al.*^[4] first reported the isolation of *C. concisus* from patients with gingivitis in 1981. Zhang *et al.*^[5] examined the presence of *C. concisus* in saliva samples obtained from healthy individuals of different age groups and found that *C. concisus* is commonly present in the human oral cavity. In that study, *C. concisus* was detected

Table 1 Detection of *Campylobacter concisus* in samples obtained from healthy individuals

Samples	Cultivation of <i>C. concisus</i>	Detection of <i>C. concisus</i> DNA by PCR
Human saliva	75%	97%-100%
Human feces	0%-2.8%	33%
Human intestinal biopsies	0%	2%-38%

Data shown in this table were obtained from references 5-10 and 17-21. To date, no studies have detected *Campylobacter concisus* (*C. concisus*) in samples obtained from healthy animals. The collective data suggest that humans are the natural host of *C. concisus* and the oral cavity is the primary colonization site. PCR: Polymerase chain reaction.

in 97% (57/59) of saliva samples collected from healthy individuals aged 3-60 years old by polymerase chain reaction (PCR) targeting 16S rRNA gene and cultured from 75% (44/59) of these saliva samples using a filtration method. A study from Petersen *et al.*^[6] also detected a high prevalence of *C. concisus* in the human oral cavity: in this study *C. concisus* was detected in 100% of saliva samples (11/11) collected from healthy individuals by a PCR targeting 16S rRNA gene. Despite its high prevalence in the human oral cavity, *C. concisus* is not a dominant oral bacterial species^[7].

In comparison to the high isolation of *C. concisus* from saliva samples, the isolation rates of *C. concisus* from fecal samples collected from healthy individuals were much lower. Using the filtration method, Engberg *et al.*^[8] isolated *C. concisus* from 2.8% (3/107) of fecal samples and Nielsen *et al.*^[9] did not isolate *C. concisus* from any of 108 fecal samples collected from healthy individuals. The low isolation rates of *C. concisus* from fecal samples suggest that the human intestinal tract of healthy individuals is a less optimal site for *C. concisus* colonization compared to the oral cavity.

To date, *C. concisus* has not been detected in any healthy animals. In a study examining the presence of *Campylobacter* species in fecal samples collected from 70 healthy pet dogs using a quantitative PCR targeting the 60 kDa chaperonin gene, Chaban *et al.*^[10] detected seven different campylobacter species but not *C. concisus*. Various campylobacter species have been detected in fecal samples collected from animals or birds, but *C. concisus* was not detected^[3,10,11]. Lynch *et al.*^[12] isolated *C. concisus* from 10% (18/185) of chicken meat and 3% of beef meat (6/186) samples. However, whether chicken and cattle are natural hosts of *C. concisus* cannot be determined from these data.

C. concisus has been detected in some animals with gastrointestinal disorders. Petersen *et al.*^[6] detected *C. concisus* in 12.5% (1/8) of saliva samples from pet cats with dental diseases by PCR targeting the 16S rRNA gene^[6]. In addition, Chaban *et al.*^[10] detected *C. concisus* in fecal samples of 9% of dogs with diarrhea (6/65).

The collective data suggest that humans are the natural host of *C. concisus*, with the human oral cavity being the primary colonization site (Table 1).

Table 2 Genetic relatedness of enteric and oral *Campylobacter concisus* strains

Enteric <i>C. concisus</i> strains from patients with IBD	Genetically related <i>C. concisus</i> strains
A strain isolated from intestinal biopsies of patient No. 1	An oral strain from patient No. 6
A strain isolated from intestinal biopsies of patient No. 3	An oral strain from patient No. 3 (identical)
A strain isolated from luminal fluid of patient No. 3	An oral strain from healthy No. 1

Data shown in this table were obtained from reference 13. The genetic relatedness was assessed based on the sequences of six housekeeping genes. These data provide evidence showing that *Campylobacter concisus* (*C. concisus*) strains colonizing the intestinal tract of patients with IBD originating from either the patient's own oral *C. concisus* strains or oral *C. concisus* strains from other individuals. IBD: Inflammatory bowel disease.

DIVERSITY OF *C. CONCISUS* STRAINS COLONIZING THE HUMAN ORAL CAVITY

C. concisus strains colonizing the human oral cavity are greatly diverse. On examination of oral *C. concisus* strains isolated from individual patients with IBD and healthy controls, it was found that *C. concisus* strains isolated from each individual had unique protein patterns on sodium dodecyl sulphate polyacrylamide gel electrophoresis^[5,13,14]. Furthermore, some individuals were colonized with multiple *C. concisus* strains in the oral cavity, with as many as three different *C. concisus* strains having been isolated from individual patients with IBD or healthy controls^[5,13,14]. A significantly higher number of patients with active IBD were colonized with multiple *C. concisus* strains in the oral cavity compared to healthy controls^[14].

COLONIZATION OF ORAL *C. CONCISUS* STRAINS IN THE INTESTINAL TRACT

It is estimated that 1-1.5 L of saliva is produced daily in humans, most of which is swallowed^[15]. Thus, the human oral cavity is a constantly available source, transporting *C. concisus* from the oral cavity along with saliva to lower parts of the gastrointestinal tract. However, as mentioned previously, both the detection of *C. concisus* by PCR and the cultivation of *C. concisus* from fecal samples were much lower than that from saliva samples, indicating that *C. concisus* routinely transported from the oral cavity to the intestines does not commonly establish colonization there.

Nevertheless, intestinal colonization of oral *C. concisus* strains does occur in some individuals. For example, in a study comparing the housekeeping genes of *C. concisus* strains isolated from intestinal biopsies of patients with IBD and controls, Ismail *et al.*^[13] found that a *C. concisus* strain isolated from intestinal biopsies of a patient with UC had housekeeping genes identical to that of an oral *C. concisus* strain isolated from the same patient, providing evidence that the oral *C. concisus* strains are in fact able to colonize the human intestinal tract (Table 2).

In addition to an individual's own oral *C. concisus*, *C.*

concisus detected in the intestinal tract may also come from a different source, most likely through materials contaminated with saliva from others. For example, in the above patient with UC, while the *C. concisus* strain isolated from intestinal biopsies had housekeeping genes identical to that of the patient's own oral *C. concisus* strain, the *C. concisus* strain isolated from the luminal fluid of this patient had a genetic relationship closely related to an oral *C. concisus* strain from a healthy control, rather than to the patient's own oral *C. concisus* (Table 2)^[13].

The human intestinal environment is not optimal for *C. concisus* colonization in general, as suggested by the low isolation rate of *C. concisus* from fecal samples of healthy individuals (Table 1). Given this, *C. concisus* intestinal colonization is most likely a short-term event in most individuals. However, with the human oral cavity as a constantly available source of *C. concisus*, repeated intestinal colonization of *C. concisus* may occur.

Whether the characteristics of a given oral *C. concisus* strain or the intestinal environment of an individual, or both, plays the major role in determining whether or not intestinal colonization of oral *C. concisus* strains will occur is currently unknown. A previous study by Haag *et al.*^[16] showed that intestinal microbiota shifts towards elevated commensal *Escherichia coli* loads abrogated colonization resistance against *Campylobacter jejuni* in mice. Currently, it is not clear whether the dysbiosis associated with IBD plays a role in *C. concisus* intestinal colonization.

PREVALENCE OF *C. CONCISUS* IN THE INTESTINAL TRACT OF PATIENTS WITH IBD AND HEALTHY CONTROLS

A number of studies have examined the prevalence of *C. concisus* in the intestinal tract of patients with IBD using PCR methods. Most of these studies detected a significantly higher prevalence of *C. concisus* DNA in patients with IBD and controls^[17-21]. The reported detection rates of *C. concisus* by PCR in enteric samples (biopsies and fecal samples) were 33%-69% in patients with IBD and 2%-38% in controls^[17-21]. Analysis of the data in these studies revealed a number of interesting findings.

Firstly, different PCR strategies affect the detection of *C. concisus* in enteric samples. This was best seen in the study conducted by Man *et al.*^[18], who compared the prevalence of *C. concisus* in fecal samples collected from 54 children with CD, 33 healthy controls and 27 non-IBD controls using two different PCR methods. The first PCR method employed campylobacter genus specific PCR (primers C412F/C1288R) and sequencing the PCR products to determine campylobacter species. The second PCR method was a nested PCR, using campylobacter genus specific PCR (primers C412F/C1288R) followed by *C. concisus* specific PCR (primers Concisus F/Concisus R). These two PCR methods yielded very different results in detection of *C. concisus* in the same samples. The prevalence of *C. concisus* in children with CD, healthy controls and non-IBD controls detected by *C. concisus* genus specific PCR

Table 3 Detection rates of *Campylobacter concisus* in fecal samples by two polymerase chain reaction methods

	Campylobacter genus PCR	Nested PCR
CD (<i>n</i> = 54)	19%	65%
Healthy controls (<i>n</i> = 33)	12%	33%
Non-IBD controls (<i>n</i> = 27)	0	37%

An example showing that different polymerase chain reaction (PCR) methods affect the detection of *Campylobacter concisus* (*C. concisus*) in intestinal samples. Data included in this table were from reference 18. Primers C412F and C1288R were used in campylobacter genus PCR. In nested PCR, PCR products amplified by primers C412F and C1288R were amplified again using *C. concisus* specific primers Concisus F and Concisus R. The nest PCR detected a significantly higher intestinal prevalence of *C. concisus* in patients with Crohn's disease (CD) compared to both healthy controls and non-inflammatory bowel disease (IBD) controls. The genus PCR detected a significantly higher intestinal prevalence of *C. concisus* in patients with CD compared to non-IBD controls, but not healthy controls.

was 19% (10/54), 12% (4/33) and zero (0/27) respectively. The nested PCR greatly increased the detection of *C. concisus* in the same cohort of samples, with the prevalence of *C. concisus* being 65% (35/54) in children with CD, 33% (11/33) in healthy controls and 37% (10/27) in non-IBD controls. The nested PCR, but not the genus specific PCR, detected a significantly higher prevalence of *C. concisus* in children with CD as compared to healthy controls^[18] (Table 3). Indeed, in studies revealing a significant difference in intestinal prevalence of *C. concisus* between patients with IBD and controls, nested PCR was used to examine all of the samples or part of the samples^[17-20].

Secondly, collection of multiple intestinal biopsies increases the detection of intestinal prevalence of *C. concisus*. A study by Mahendran *et al*^[20] showed that in comparison to the collection of one biopsy, the collection of four biopsies from each individual greatly increased the detection of *C. concisus* (Figure 2).

Thirdly, despite the increased prevalence of *C. concisus* detected by PCR in the intestinal tract of patients with IBD, the isolation rates of *C. concisus* from intestinal biopsies of patients with IBD were low (3%-7.7%)^[17,20,21]. This suggests that *C. concisus* detected in most of the enteric samples were at a low number or in a nonculturable state.

ADVERSE EFFECTS OF *C. CONCISUS* ON INTESTINAL EPITHELIAL CELLS

A number of adverse effects of *C. concisus* on intestinal epithelial cells have been described. Using *in vitro* cell culture models (Caco2 cells or HT-29 cells), epithelial adhesion and invasion, damage of the barrier function and up-regulation of Toll-like receptors-4 expression by *C. concisus* have been reported. Different strains showed varying degrees of ability to induce such adverse effects^[13,22-25]. The underlying molecular mechanisms responsible for these effects have not yet been investigated.

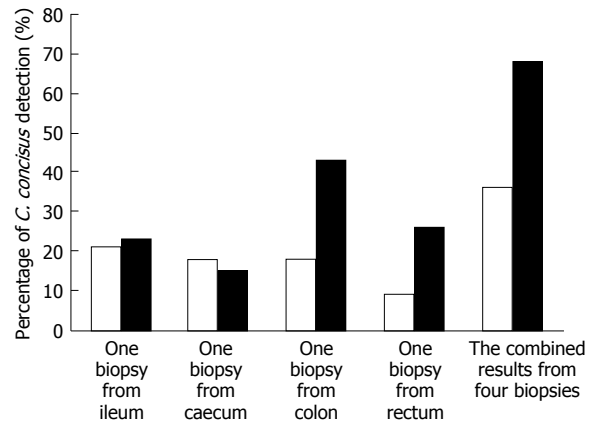


Figure 2 An example showing that collection of multiple intestinal biopsies increases the detection of intestinal prevalence of *Campylobacter concisus*. Data included in Figure 2 were from reference 20. In both patients with inflammatory bowel disease (IBD) (right column) and healthy controls (left column), the detection of intestinal prevalence of *Campylobacter concisus* (*C. concisus*) was greatly increased using four biopsies as compared to using one biopsy. In healthy controls, *C. concisus* detection rates in a single biopsy collected from ileum, caecum, colon and rectum were 21%, 18%, 18% and 9% respectively. If results from the four biopsies were used in determining the intestinal prevalence of *C. concisus*, the intestinal prevalence of *C. concisus* in healthy controls was 36%. Similarly, in patients with IBD, *C. concisus* detection rates in a single biopsy collected from ileum, caecum, colon and rectum were 23%, 15%, 43% and 26% respectively, and the intestinal prevalence of *C. concisus* in patients with IBD was 68% when four biopsies were taken into consideration.

C. CONCISUS ZOT GENE AND IBD

Mahendran *et al*^[14] examined the prevalence of *zot* gene in 56 oral *C. concisus* strains isolated from saliva of 19 patients with IBD and 20 healthy controls. This study showed that 30% (17/56) of the oral *C. concisus* strains carried the *zot* gene. The *zot*-positive *C. concisus* strain was present in 55% (6/11) of patients with active IBD and 40% (8/20) of healthy controls. Some IBD patients with active disease 18% (2/11) were colonized with multiple *zot*-positive *C. concisus* strains in the oral cavity. Interestingly, polymorphic forms of the *C. concisus* *zot* gene resulting in the substitution of valine at amino acid position 270 were found to be associated with active IBD.

The *zot* gene was first discovered in *Vibrio cholerae* where it is carried by a filamentous prophage^[26,27]. The *zot* gene in *V. cholerae* is required for phage production; a *V. cholerae* strain with *zot* gene mutation did not produce phage particles into the culture supernatant^[28]. The *V. cholerae* Zot toxin was shown to increase intestinal permeability and to be associated with mild to moderate diarrhea^[26,29,30].

Previous studies have found a human intestinal Zot analogue, namely zonulin, which is a physiological regulator that increases the intestinal permeability^[31,32].

C. CONCISUS ZOT GENE IS A COMPONENT OF A CHROMOSOMALLY INTEGRATED PUTATIVE PROPHAGE

Stothard *et al*^[33] established a visual database of bacterial

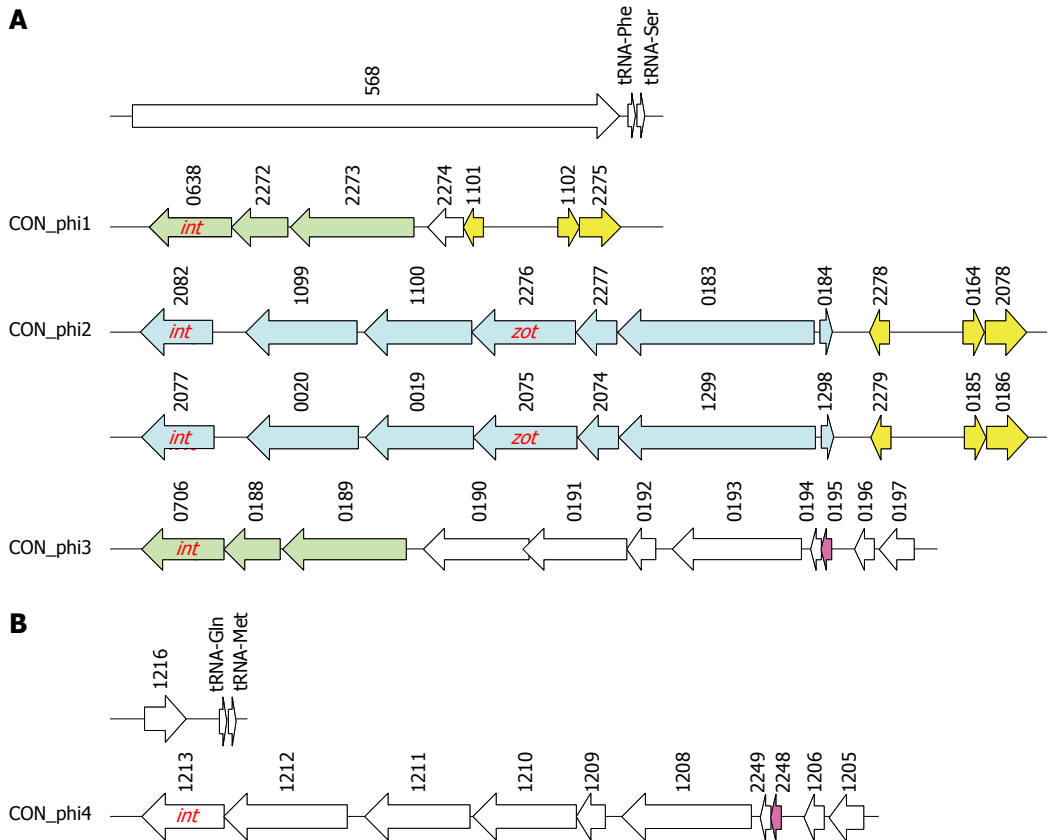


Figure 3 Genetic structures of putative prophage identified in *Campylobacter concisus* strain 13826. A: Multiple prophages at nucleotide position between 1576686 and 1614075; B: One putative prophage at nucleotide position between 939158 and 946901. Identical genes were indicated with the same color. *int*: A gene encodes phage integrase; *zot*: Zonula occludens toxin gene. The numbers are the locus tags.

chromosome maps in which *C. concisus* strain 13826 (Accession No. CP000792. 1) was included. Stothard *et al*^[33] identified a region from nucleotide position 1576683 to 1615449 (38767 bp) in the genome of *C. concisus* strain 13826 as an “incomplete prophage”. In this region, 39 genes with open reading frames were identified, with four of these genes encoding integrases and 10 genes encoding phage-like proteins^[33].

The genetic structures of this “incomplete prophage” region are shown in Figure 3A and the proteins encoded by genes in this region are shown in Table 4. We compared the genes within this region using publicly available softwares^[34,35]. A number of genes that have identical nucleotide sequences, including CCC13826_1099, CCC13826_0020, CCC13826_1102, CCC13826_0164, CCC13826_0185, CCC13826_2082 and CCC13826_2077 were annotated with identical protein names.

Interestingly, the region that was considered as an “incomplete prophage” by Stothard *et al*^[33] turned out to be four putative prophages, each beginning with a phage integrase (Figure 3 and Table 4). The first prophage had a genome size of 5.2 kb, which contained seven protein-encoding genes. The second prophage and third prophage were identical, each having a genome size of 9.6 kb consisting of 10 protein-encoding genes. The fourth prophage contained 11 protein-encoding genes with a genome size of 8.6 kb. We named the first prophage

CON_phi1, the second prophage and third prophage CON_phi2 and the fourth prophage CON_phi3. The *zot* gene is a component of CON_phi2. Comparison of the proteins encoded by genes in CON_phi2 with that encoded by genes in CTX phage, the phage that carries the *zot* gene in *V. cholera*, did not show high similarities except for the Zot protein (data not shown), suggesting the CON_phi2 is a previously uncharacterised prophage.

A study by Kaakoush *et al*^[36] found that two hypothetical proteins encoded by CCC13826_0191 and CCC13826_1210 have 47% and 46% similarity respectively to *C. concisus* Zot. Here we found that CCC13826_0191 is a gene of CON_phi3 and CCC13826_1210 is a gene of an additional putative prophage, which was named CON_phi4. A number of genes in CON_phi3 and CON_phi4 had high similarities, however, CON_phi4 did not contain the gene that corresponding to CCC13826_0188 in CON_phi3 (Table 5).

IDENTIFICATION OF CONSERVED MOTIFS SHARED BY *C. CONCISUS* ZOT AND ZONULIN/ZOT RECEPTOR BINDING DOMAINS

Kaakoush *et al*^[36] compared Zot sequences in *C. concisus* strain 13826 and *V. cholerae* strain 86015 and reported that

Table 4 Proteins encoded by putative prophages in *Campylobacter concisus* strain 13826

Nucleotide position	Locus tag	Encoded proteins	Size (aa)
1576686..1581986	CCC13826_0568	Hypothetical protein	1766
1582376..1583269	CCC13826_0638	Phage integrase	297
1583269..1583880	CCC13826_2272	Glutathionylspermidine synthase family	203
1583895..1585235	CCC13826_2273	Bacteriophage replication gene A protein	446
1585467..1585853	CCC13826_2274	Protein yitk	128
1585894..1586115	CCC13826_1101	Phosphonate uptake transporter	73
1586807..1587043	CCC13826_1102	Sensory box protein	78
1587044..1587496	CCC13826_2275	Hypothetical protein	150
1587598..1588506	CCC13826_2082	Phage integrase	302
1588755..1589966	CCC13826_1099	Putative phage replication protein A	403
1590015..1591184	CCC13826_1100	ATP binding protein (ABC) transporter	389
1591228..1592352	CCC13826_2276	Zonula occludens toxin (Zot) family protein	374
1592354..1592800	CCC13826_2277	Hypothetical protein	148
1592797..1594923	CCC13826_0183	Hypothetical protein	708
1594955..1595095	CCC13826_0184	Hypothetical protein	46
1595477..1595698	CCC13826_2278	Phosphonate uptake transporter	73
1596412..1596648	CCC13826_0164	Sensory box protein	78
1596649..1597101	CCC13826_2078	Hypothetical protein	150
1597203..1598111	CCC13826_2077	Phage integrase	302
1598360..1599571	CCC13826_0020	Putative phage replication protein A	403
1599620..1600789	CCC13826_0019	ABC transporter	389
1600833..1601957	CCC13826_2075	Zot family protein	374
1601959..1602405	CCC13826_2074	Hypothetical protein	148
1602402..1604528	CCC13826_1299	Hypothetical protein	708
1604560..1604700	CCC13826_1298	Hypothetical protein	46
1605082..1605303	CCC13826_2279	Phosphonate uptake transporter	73
1606017..1606253	CCC13826_0185	Sensory box protein	78
1606254..1606706	CCC13826_0186	Hypothetical protein	150
1606808..1607701	CCC13826_0706	Phage integrase	297
1607701..1608312	CCC13826_0188	Glutathionylspermidine synthase family	203
1608327..1609667	CCC13826_0189	Bacteriophage replication gene A protein	446
1609899..1611041	CCC13826_0190	Type II and III secretion system protein	380
1610992..1612116	CCC13826_0191	Hypothetical protein	374
1612118..1612438	CCC13826_0192	Hypothetical protein	106
1612537..1613946	CCC13826_0193	Hypothetical protein	469
1613956..1614075	CCC13826_0194	Alkyl hydroperoxide reductase	39
1614090..1614209	CCC13826_0195	Hypothetical protein	39
1614489..1614707	CCC13826_0196	Hypothetical protein	72
1614801..1615178	CCC13826_0197	Hypothetical protein	125

aa: Amino acids.

the biological active domain (FCIGRL) previously found in *V. cholerae* Zot was not found in *C. concisus* Zot. In this review, we compared the sequence of *C. concisus* Zot with human zonulin receptor binding domain and *V. cholerae* Zot receptor binding domain previously reported^[32,37]. Interestingly, we found that *C. concisus* Zot shares conserved motifs with both the human zonulin receptor binding domain and the *V. cholerae* Zot receptor binding domain (Table 6). These data suggest that *C. concisus* Zot may increase intestinal permeability using a mechanism that is similar to the human zonulin and *V. cholerae* Zot, affecting the tight junctions through proteinase activated receptor 2 activation^[37,38]. The motif “GRFLSYHG” is located at amino acid position 123-130 in *C. concisus* Zot, which was found in all oral *zot*-positive *C. concisus* strains that we previously isolated as well as in the *C. concisus* strain 13826^[14]. The polymorphisms of *C. concisus* *zot* gene that Mahendran *et al*^[14] previously detected were not in the receptor binding domain, suggesting that these polymorphisms may impact on the function of *C. concisus* Zot, if there is

any, using a different mechanism rather than affecting the binding of *C. concisus* Zot to the receptor.

INCREASED INTESTINAL PERMEABILITY IN PATIENTS WITH IBD

Increased intestinal permeability is a feature of both CD and UC^[39-43]. While epithelial cell death and proinflammatory cytokines may damage the intestinal epithelial barrier during active disease, evidence shows that increased intestinal permeability may precede the initial onset or relapse of IBD. An early study from Hollander *et al*^[39] reported that increased intestinal permeability was detected not only in patients with CD but also in their healthy relatives. A family history of IBD is a known risk factor for IBD^[44]. Irvine *et al*^[41] reported that an individual with a family history of CD had elevated intestinal permeability eight years prior to the onset of clinical symptoms and diagnosis of CD. Wyatt *et al*^[43] measured the intestinal

Table 5 The similarity of proteins in CON_phi3 and CON_phi4

CON_phi3	CON_phi4	Similarity ¹
CCC13826_0706	CCC13826_1213	23.23%
CCC13826_0188		
CCC13826_0189	CCC13826_1212	93.72%
CCC13826_0190	CCC13826_1211	98.95%
CCC13826_0191	CCC13826_1210	92.25%
CCC13826_0192	CCC13826_1209	92.03%
CCC13826_0193	CCC13826_1208	58.90%
CCC13826_0194	CCC13826_2249	100%
CCC13826_0195	CCC13826_2248	97.44%
CCC13826_0196	CCC13826_1206	61.11%
CCC13826_0197	CCC13826_1205	95.24%

¹Similarity is the percentage of identical amino acids.

permeability in patients with quiescent CD and found that those with increased intestinal permeability were at a significantly higher risk of clinical relapse. These data suggest that increased intestinal permeability occurred prior to the onset and relapse of the disease may be a possible aetiological factor of IBD.

C. CONCISUS ZOT: A POTENTIAL TRIGGER OF IBD THROUGH CAUSING PRIMARY BARRIER DEFECT

The human zonulin and *V. cholerae* Zot toxin are known to increase intestinal permeability through affecting the tight junctions^[31,32,37]. In this review, we found that *C. concisus* Zot has conserved motifs shared by the zonulin/Zot binding receptor domains. Given this, it is very likely that *C. concisus* Zot also affects the tight junctions.

Based on the information obtained from previous publications and the analysis that we have performed in this review, we propose a mechanism by which *C. concisus*, an oral bacterium, may trigger the onset or relapse of IBD: that some oral *C. concisus* strains acquire *zot* gene from a virus (prophage). With the human oral cavity as the reservoir of *C. concisus*, repeated intestinal colonization of *C. concisus* and release of *C. concisus* Zot due to prophage induction may occur, which is likely to result in a prolonged primary epithelial barrier defect and translocation of macromolecule such as luminal microbes and their products. In genetically susceptible individuals, this may trigger the development of IBD.

Damage to the intestinal epithelial tight junctions may also lead to the development of diarrhea. Indeed, in addition to its association with IBD, *C. concisus* has been frequently isolated from non-IBD-related diarrheal stool samples^[9,45,46].

If some oral *C. concisus* strains are indeed involved in the development of human IBD, the question as to why the lesions of IBD occur more often in the intestinal tract rather than in the oral cavity arises. One explanation is that the virulence factors that are associated with IBD are more often expressed in the intestinal tract rather

Table 6 Motifs shared by *Campylobacter concisus* zonula occludens toxin and zonulin/zonula occludens toxin receptor binding domains

<i>C. concisus</i> Zot ¹	GRF LSYHG
Human adult intestine zonulin	GGXL
Human fetal intestine zonulin	GGVLVQPG
<i>C. concisus</i> Zot ²	GRF LSYHG
<i>V. cholerae</i> Zot	FCIGRLCVQDG

¹*Campylobacter concisus* zonula occludens toxin (Zot) and zonulin binding domain shares a motif (bold letters): Non-polar G, variable, non-polar, non-polar L, variable, polar, variable and non-polar G; ²*C. concisus* Zot and *Vibrio cholerae* Zot shares a motif (bold letters): non-polar G, basic polar R, non-polar, non-polar, variable, polar, variable and non-polar G. Comparison of *C. concisus* Zot and zonulin/Zot receptor was performed in this review. The amino acid sequences of human intestinal zonulin and *V. cholerae* Zot were obtained from reference 32.

than in the oral cavity. For example, the expression of *C. concisus* Zot may require induction of prophage from the *C. concisus* genome. As prophage induction usually occurs when bacterial cells are under stressful conditions^[47], the fact that *C. concisus* uses the human oral cavity as its primary colonization site suggests that the oral cavity is not a stressful site for *C. concisus*. However, as the *C. concisus* travels to the more hostile lower parts of the gastrointestinal tract, the stressful environment may trigger the induction of *C. concisus* prophage.

Another possible factor that may reduce the pathogenic effect of *C. concisus* Zot in the oral cavity is that the epithelium in the oral cavity is a stratified squamous epithelium, either keratinized or non-keratinized^[48]. In contrast, the intestinal epithelium is a simple columnar epithelium^[48]. The impact on permeability caused by Zot, even it is expressed in the oral cavity, in multiple layers of squamous epithelium may not be as evident as that in the single layered columnar epithelium.

C. CONCISUS ZOT: A POTENTIAL ENVIRONMENTAL FACTOR CONTRIBUTING TO THE INCREASED RISK OF IBD IN INDIVIDUALS WITH A FAMILY HISTORY OF IBD

A family history of IBD is a risk factor for developing IBD^[44]. In addition to genetic factors, environmental factors have been shown to be involved in the increased incidence of IBD in members with a family history of this disease^[49,50]. We suggest that *C. concisus* Zot is one such factor. This suggestion is based on the findings that the higher numbers of the relatives of patients with IBD have increased intestinal permeability and that some oral *C. concisus* strains carry the *zot* gene that encodes a toxin known to promote this^[14,39,41]. This hypothesis remains to be further assessed by examining the correlation between colonization of *zot*-positive *C. concisus* strains and the increased intestinal permeability in family members of patients with IBD.

CONCLUSION

The evidence presented in this review shows that some *C. concisus* strains colonizing the human oral cavity acquired *zot* gene from a virus (prophage). We are currently examining the biologic activities of *C. concisus* *Zot*, the expression of *Zot* in *zot*-positive *C. concisus* strains isolated from patients with IBD and controls as well as the presence of *C. concisus* *Zot* in the oral cavity and intestinal tract of patients with IBD and controls, which will provide further information in understanding the role of *C. concisus* *Zot* in IBD and other human diseases.

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Immunosuppressive therapies for inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) is comprised of Crohn's disease and ulcerative colitis, both chronic inflammatory intestinal disorders of unknown etiology characterized by a waxing and waning clinical course. For many years, the drug therapy was limited to sulfasalazine and related aminosalicylates, corticosteroids and antibiotics. Studies suggesting that the pathophysiology of these disorders relates to a dysregulated, over-active immune response to indigenous bacteria have led to the increasing importance of immunosuppressive drugs for the therapy of IBD. This review details the mechanisms of action, clinical efficacy, and adverse effects of these agents.

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Key words: Crohn's disease; Ulcerative colitis; Inflammatory bowel disease; Immunosuppressives; Tumor necrosis factor inhibitors

Core tip: This manuscript reviews the current status of immunosuppressive therapy for inflammatory bowel disease. It describes the mechanism of action, clinical efficacy and adverse effects of immunomodulators including azathioprine, 6-mercaptopurine, methotrexate, and cyclosporine and biologics including anti-tumor necrosis factor (TNF) agents and adhesion molecule inhibitors. It emphasizes the role of azathioprine, 6-mercaptopurine, and methotrexate in the long-term maintenance of Crohn's disease, the utility of cyclosporine in severe refractory ulcerative colitis and the unique role of anti-TNF agents in the remission induction and maintenance of difficult to treat patients with Crohn's disease and ulcerative colitis.

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INTRODUCTION

Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis, both chronic inflammatory disorders of the gastrointestinal tract, characterized by a relapsing and remitting course^[1]. In the United States, the incidence of Crohn's disease is estimated to be between 6-8 per 100000, with a prevalence of 100-200 per 100000. The incidence of ulcerative colitis is estimated to be between 9-12 per 100000, with a prevalence of 205-240 per 100000^[2]. IBD is associated with high health care costs, and can result in a significant quality of life burden. Unlike ulcerative colitis, which is limited to the mucosa, Crohn's disease typically causes transmural inflammation, and can result in stricturing and penetrating complica-

tions. The goals of therapy are two-fold, and include induction and maintenance of remission, and avoidance of complications. Remission has traditionally been defined as the achievement of clinical remission, but a more recent trend has been towards achieving mucosal healing, or deep remission^[3]. These goals are achieved through lifestyle modification, medical management, and surgery when necessary. Though the underlying etiology of these diseases remains poorly understood, it is thought that Crohn's disease and ulcerative colitis are driven by an inappropriate immune inflammatory response to gut microbes, in a genetically predisposed host^[4]. The role of immunity is reflected in the focus on immunosuppressive medications in inducing and maintaining remission.

Though historically reserved for patients failing "conventional" therapies (such as 5-ASAs, antibiotics, and in some cases, steroids), immunosuppressive therapies such as immunomodulators and biologics are being used earlier in an attempt to alter the natural history of IBD^[5]. Though corticosteroids are among the oldest and most effective therapies in IBD^[6], their side effect profile limits their appeal^[5], and maintenance of a steroid-free remission has become a key tenet of the management of IBD.

The purpose of this review is to summarize the available immunosuppressive options for the medical management of IBD.

IMMUNOMODULATOR THERAPIES

Immunomodulators include thiopurines [6-mercaptopurine (6-MP) and azathioprine (AZA)], methotrexate (MTX), and cyclosporine (CSA).

Thiopurine analogues

Crohn's disease and ulcerative colitis: Though for many years these medications have been widely used as steroid-sparing agents for the maintenance of remission in moderate-to-severe IBD, the data overall supporting their efficacy are limited and often contradictory, particularly in ulcerative colitis^[7,8]. Older data have shown that thiopurines can be particularly effective in the long-term management of peri-anal and fistulizing Crohn's disease^[9]. These drugs are not suited to the induction of remission^[7], given a mean response time of 2-3 mo^[10]. None the less, their use is widespread, and their role on the treatment pyramid well established^[1,11].

6-MP and AZA are thought to act by inhibiting lymphocyte proliferation *via* the incorporation of active drug metabolites into cellular nucleotides, which likely results in anti-inflammatory effects through suppression of T cell function and natural killer cell activity^[12,13]. AZA is the active pro-drug of 6-MP, and both are similarly converted to their therapeutic end-product, 6-thioguanine (6-TG), to the inactive metabolite, 6-thiouric acid, by xanthine oxidase, and to the hepatotoxic metabolite 6-methylmercaptopurine (6-MMP) by the thiopurine methyltransferase (TPMT) enzyme^[1,13]. Lower doses may be needed in patients with intermediate TPMT enzyme

activity in order to avoid leukopenia caused by high levels of 6-TG, and neither drug can be used at all in the 0.3% of the population deficient in the enzyme, due to the risk of life-threatening toxic complications^[10]. Xanthine oxidase inhibitors can be used to boost response in patients who preferentially shunt towards 6-MMP. Testing for TPMT and measurement of both therapeutic and toxic metabolite levels are readily available in the United States, and can be used as an adjunct to routine monitoring of blood counts and liver function tests.

Dose-independent, or hypersensitivity, reactions have been described with use of 6-MP/AZA, and include hepatitis, pneumonitis, arthritis, and fever. Perhaps the most serious dose-independent reaction is pancreatitis, which can occur in approximately 4% of treated patients^[14]. The reactions usually occur early in the course of therapy, and typically resolve with discontinuation of the medication. Minor side effects such as nausea, vomiting, and flu-like illness are possible, though thiopurines are typically well tolerated in 75% of patients using them^[15]. Serious opportunistic infections are possible, as with any immunosuppressant, but uncommon^[7,10]. With regards to the development of cancer, and in particular lymphoma, following the use of these therapies, a recent meta-analysis^[16] concluded that IBD-patients on thiopurines have a 4-fold increased risk of lymphoma, but whether this increase was due to the medication or the underlying disease could not be established. These data were comparable to those from the CESAME group^[17]. Moreover, this level of risk was well below what was deemed necessary to impart significant reduction in quality-adjusted life expectancy compared to other treatment strategies^[11,18]. Ongoing and past exposure to thiopurines has been shown to significantly increase the risk of non-melanomatous skin cancers^[19], and as such patients on these therapies should be advised to use adequate sun protection and have routine skin examinations.

There has been particular concern with regards to the association between hepatosplenic T cell lymphoma (HSTCL) and thiopurine use. HSTCL is a rare but fatal lymphoma, which appears to occur more frequently in patients with IBD as compared to the general population, though the absolute risk remains very low^[20]. The risk of HSTCL appears to be higher in patients receiving thiopurines (both for IBD or for other reasons), and especially in those with long-term exposure^[20]. A 2011 review^[20] of all cases of HSTCL in IBD identified 2 other factors, male gender and age 10-35, as being associated with the development of HSTCL. Though combination therapy with thiopurines and anti-tumor necrosis factors (TNFs), particularly in this cohort of young males with IBD, has been postulated to portend an even high risk for developing HSTCL, this has not actually been demonstrated and is more theoretical, particularly since there were no cases of IBD patients treated with anti-TNF monotherapy who developed HSTCL. None the less, the authors concluded that combination therapy should be used in patients with IBD only when a clear benefit was expected^[20]. Combination therapy is discussed in more detail below.

Methotrexate

Crohn's disease: MTX is a folic acid antagonist, and is thought to act by interrupting DNA synthesis and increasing adenosine^[21], and by inhibiting interleukin (IL)-1 and suppressing T cell function. Its role in the management of IBD is much less well established than that of thiopurines. There is evidence to support the use of parenteral MTX in induction and maintenance of remission in refractory, steroid-dependent Crohn's disease^[8]. There are no convincing data to support its use in ulcerative colitis - the few studies that exist are limited both by size, quality, and the fact they used lower doses than what was shown to be effective in Crohn's disease (15 mg/wk *vs* 25 mg/wk)^[8].

MTX is thought to be safe and tolerable. Nausea can occur in 15% of patients, but can typically be prevented with the co-administration of folate 1 mg/d. Leukopenia, hepatotoxicity, hypersensitivity pneumonitis, and opportunistic infections have been reported but are uncommon. MTX is teratogenic and should never be used in pregnant women or those contemplating pregnancy^[8,13].

Cyclosporine

Ulcerative colitis: CSA is a calcineurin inhibitor, and is thought to act by decreasing pro-inflammatory lymphokines by inhibiting their antigen-induced secretion through the binding of calcium calmodulin-dependent protein phosphatase calcineurin^[22].

The data with respect to the use of calcineurin inhibitors in IBD are very limited. CSA has proven to have promise in the induction of remission of refractory ulcerative colitis, but the data with regards to its use in Crohn's disease are less convincing. Oral cyclosporine has not consistently been shown to be effective in the induction of remission of Crohn's disease (though one study showed a modest effect at higher doses)^[23-25]. Uncontrolled data demonstrated some effectiveness in treating fistulizing Crohn's disease with parenteral CSA^[26]. Studies in patients with refractory ulcerative colitis have shown that when used in the acute setting, as a bridge to thiopurine maintenance, CSA can be valuable in inducing remission and delaying or avoiding colectomy^[27].

CSA is generally considered to be less safe than other IBD therapies, because of the risk of serious side effects, such as anaphylaxis, seizure, pneumocystis carinii pneumonia, and permanent nephrotoxicity^[10]. Moreover, ease of use is limited by the need for close monitoring of drug levels due the narrow gap between the therapeutic and toxic ranges^[27]. As such, CSA is typically reserved as a rescue agent for severe, refractory disease.

BIOLOGICS

Perhaps the most significant advance in the treatment of IBD has been the introduction of anti-TNF-alpha monoclonal antibodies, and subsequent biologic therapies, both for Crohn's disease and ulcerative colitis.

Infliximab

Infliximab (IFX) is a chimeric, monoclonal antibody

(75% human, 25% mouse) that targets and binds to TNF-alpha, a potent pro-inflammatory cytokine thought to play a role in gut inflammation^[28].

Crohn's disease: IFX was initially released in 1998 for the treatment of moderate-to-severe and fistulizing Crohn's disease, after it was shown to induce remission in a small, uncontrolled study of steroid-refractory patients^[28]. These findings were later replicated in a larger, placebo-controlled study^[29], where IFX was shown to induce remission in one third of steroid-refractory patients with luminal Crohn's. The landmark study that guides our current use of IFX as an induction and maintenance medication in luminal Crohn's disease is known as ACCENT I, which showed that the 58% of patients considered to be responders to an initial IFX infusion were more likely to have a sustained remission after 1 year when maintained on q8 week infusions after an initial loading period^[30]. Subsequent research has shown a benefit of long-term therapy at 5-years^[31].

IFX has also been shown to be effective in treating fistulizing Crohn's disease, resulting in both complete closure of draining abdominal and perianal fistulae at 3 mo in 55% of patients receiving IFX 5 mg/kg (compared to 13% of patients receiving placebo)^[32], and in the long-term maintenance of remission of fistulizing Crohn's disease in 36% of patients (compared to 19% in the placebo group) at week 54 follow-up (ACCENT II)^[33]. A recent small-scale retrospective study has shown IFX in combination with antibiotics to be safe and effective in treating phlegmons^[34].

Ulcerative colitis: IFX has also been shown to have benefit in the treatment of ulcerative colitis, specifically when refractory to conventional therapies. The ACT-1 and ACT-2 trials^[35] have shown that patients with moderate-to-severe active ulcerative colitis refractory to conventional treatment were more likely to have clinical response at weeks 8, 30, and 54 in the IFX group compared to placebo, and are less likely to have undergone colectomy by week 54^[36]. Though some have argued for the earlier implementation of IFX therapy in less severe (*i.e.* moderate) ulcerative colitis^[37], the use of IFX in ulcerative colitis has typically been reserved as third-line or rescue therapy.

Safety and tolerability: IFX is generally considered to be safe and tolerable, however a risk benefit analysis should be undertaken when considering its use given the potential for serious complications. Using the ACCENT I trial data^[30] as a fairly typical profile, 32% of patients were found to have had an infection requiring treatment by week 54, including 1 case of tuberculosis and 2 deaths from sepsis (out of 2863 treated patients). Development of a lupus-like syndrome was described though very rare, though the development of ANA and anti-ds DNA antibodies more common (up to 56% and 34% respectively). Antibodies to infliximab were detected in 14% of patients, and infusion reactions were much more common

in this group (16% *vs* 4%-6%). Infusion reactions were typically mild, and required discontinuation of drug in less than 1% of cases. Concomitant use of steroids and immunomodulators decreased the risk of infusion reaction. There was a 1% rate of development of malignancy, including lymphoma and non-melanomatous skin cancers. It is worth noting that data from the TREAT registry^[38], a prospective analysis looking at the safety of IFX and other medications for Crohn's disease, taking into account confounding factors such as disease severity and use of other medications, showed that the risk of serious infection and death was no greater in patients using IFX *vs* immunomodulators, and the overall incidence comparable to that among all patients with Crohn's disease. In fact, the only independent risk factor for serious infection and death that emerged was the use of prednisone, and for serious infection alone was narcotics. Similarly though there does appear to be an increased risk of lymphoma among patients with IBD using IFX, this risk has not been quantified, and the role of confounding factors not fully understood^[11].

Adalimumab

Adalimumab (ADA) is a fully humanized, recombinant monoclonal antibody against anti-TNF- α , and thus immunogenicity and the formation of antibodies is theoretically lower. Unlike IFX, ADA is administered subcutaneously. The safety profile of ADA is similar to that of IFX, which was discussed previously.

Crohn's disease: ADA has been shown to be effective in inducing^[39] and maintaining^[40] remission in IFX-naïve patients with moderate-to-severe Crohn's disease, those with loss of response to IFX^[41], and in patients with fistulizing Crohn's disease^[42]. Initial response rates are comparable between ADA and IFX - approximately one-third of naïve patients will achieve remission^[30,39]. The effects with regards to healing fistulas were more robust for IFX^[32,42]. Rates of mucosal healing in Crohn's disease were comparable with ADA and IFX^[43]. Rates of antibody formation were significantly less compared to IFX^[30,39].

Ulcerative colitis: ADA has also been shown to induce^[44] and maintain^[29] remission in patients with moderate-to-severe ulcerative colitis who have been refractory to conventional therapy with steroids, immunomodulators, or anti-TNFs, though the efficacy of ADA was lower in those who were not anti-TNF naïve.

Certolizuman pegol

Crohn's disease: Certolizuman pegol (CZP) is a humanized pegylated Fab fragment of an anti-TNF- α antibody. Because it does not contain an Fc portion like other monoclonal antibodies (such as IFX and ADA), CZP does not induce antibody-dependent cellular cytotoxicity. It is administered subcutaneously. It is only approved for the treatment of moderate-to-severe Crohn's disease, in

patients with inadequate response to conventional therapies, and only readily available in the United States, Russia, and Switzerland. It has been shown to have a modest improvement in response and remission rates in moderate-to-severe Crohn's disease as compared to placebo^[45,46], as well as with regards to fistulizing disease^[47]. Rates of antibody development were comparable to those with IFX^[30]. Since the lack of an Fc portion prevents the active transport of CZP across the placenta, there has been some preference for its use in women of childbearing age, however timing of administration of ADA and IFX in the third trimester can be manipulated to reduce drug concentrations in the newborn^[48]. It is generally advocated that biologic agents not be changed solely for this reason.

Natalizumab

Crohn's disease: Natalizumab (NZA) is a selective adhesion-molecule inhibitor. It is a humanized monoclonal IgG4 antibody against α -4-integrins, which are selectively involved in leukocyte transfer across the gut and brain. It has been approved for the treatment of moderate-to-severe Crohn's disease in patients who have been refractory to conventional therapies. Though initial studies looking at NZA in Crohn's disease were less promising^[49], more recent, albeit smaller studies have suggested that it may be effective in induction and maintenance of remission in moderate-to-severe Crohn's disease^[50], and in particular in patients who have lost response to anti-TNF therapies^[51].

Use of NZA has been limited due to its association with progressive multifocal leukoencephalopathy (PML), a devastating demyelinating CNS infection caused by the reactivation of JC virus^[52]. Because of this, it can only be prescribed in the United States through a restricted distribution program. JC virus antibody testing is available, but its use controversial as a screening tool in patients at high risk of developing PML. The majority of normal individuals are seropositive for JC virus, and any immunosuppressed patients are at risk for *de novo* infection. Using JC viuria as a marker for latent infection with high risk of reactivation is promising, but more research is needed^[53].

Promising therapies

Golimumab: Golimumab is a fully human anti-TNF therapy, administered subcutaneously. It was recently approved in the United States for treatment of patients with refractory ulcerative colitis based on phase 2 and phase 3 studies showing efficacy over placebo in induction and maintenance of remission. Further studies are needed before use of this medication becomes more widespread^[54,55].

Ustekinumab: Ustekinumab is a fully human IgG1k monoclonal antibody that blocks biologic activity of IL-12 and IL-23, an inflammatory pathway thought to be linked to the pathogenesis of Crohn's disease^[56]. A phase 2b clinical trial was recently published, and demonstrated improved rates of induction response to therapy among primary and secondary anti-TNF non-responders

compared to placebo, however failed to demonstrate significant improvements over placebo in actual induction of remission^[56]. None the less, phase 3 data have not yet been published, and this drug remains promising.

Vedolizumab: Vedolizumab is an investigational, humanized monoclonal antibody that selectively inhibits migration of lymphocytes into the gut by exclusively targeting alpha-4-beta-7 integrin. By being more highly selective than other anti-integrin therapies, specifically NTZ, vedolizumab is not thought to carry the same risk of PML, though long-term data are extremely limited^[57]. Though phase 2 data demonstrated a positive trend, vedolizumab was not shown to induce clinical response in Crohn's disease^[58]. Results were much more favourable in ulcerative colitis, where vedolizumab was found to be superior to placebo in inducing and maintaining remission^[59], however more studies are needed.

Combination therapy

Therapy with anti-TNF-alpha antibodies and other biologics is limited by loss of efficacy and antibody formation to the drug, underscoring the need for further research and development of novel therapies. Concomitant use of immunomodulators has been shown to decrease antibody formation and boost longevity of biologic medications. The landmark SONIC trial^[60] compared efficacy and safety of IFX and ADA alone *vs* in combination for Crohn's disease. The primary end-point of corticosteroid-free remission at week 26 was achieved by approximately 56% of patients in the combination group, *vs* 44% and 30% in the IFX and AZA groups, respectively, and this significant difference persisted through week 50. There were also significantly higher rates of mucosal healing in the combination group, without any significant increase in infections.

With respect to ulcerative colitis, the UC SUCCESS trial data, available only in abstract form to date, demonstrated superiority of IFX and AZA compared to monotherapy with either agent in inducing remission, but did not show benefit of combination therapy over IFX alone in achieving mucosal healing. This cohort was only followed for 8 wk, so no conclusion can be drawn with respect to maintenance of remission^[61].

CONCLUSION

Though the underlying genetic and molecular pathways responsible for the development and severity of IBD remain poorly understood, the therapeutic focus, particularly for more advanced disease, has been on immunosuppressive medications. The goal of therapy remains maintenance of a steroid-free remission, though striving for a deep remission with mucosal healing is becoming more standard. Advances in the understanding of the molecular basis of Crohn's and ulcerative colitis have led to the development of promising new biologic therapies, which will likely be studied further both as monothera-

peutic agents, and for use in combination with immunomodulators.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Management of intestinal failure in inflammatory bowel disease: Small intestinal transplantation or home parenteral nutrition?

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Core tip: In this review we describe and compare the principal options for the management of intestinal failure in patients with inflammatory bowel disease: home parenteral nutrition and intestinal transplantation. We describe patient survival, complications and quality of life considerations that influence individualised decision-making between approaches. As survival from transplantation improves, decision-making is likely to change.

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Abstract

Inflammatory bowel disease and Crohn's disease in particular, is a common cause of intestinal failure. Current therapeutic options include home parenteral nutrition and intestinal transplantation. For most patients, home intravenous therapy including parenteral nutrition, with a good probability of long-term survival, is the favoured choice. However, in selected patients, with specific features that may shorten survival or complicate home parenteral nutrition, intestinal transplantation presents a viable alternative. We present survival, complications, quality of life and economic considerations that currently influence individualised decision-making between home parenteral nutrition and intestinal transplantation.

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INTRODUCTION

Intestinal failure (IF) may result from obstruction, dysmotility, surgical resection, congenital defect or disease-associated loss of absorption^[1]. It is characterized by the inability to maintain protein-energy, fluid, electrolyte and/or micronutrient balance^[1]. Three categories exist: types 1, 2 and 3^[2]. Type 1 generally occurs post-operatively and is self-limiting [such as a patient developing an ileus, requiring short-term parenteral nutritional (PN) support for days or even weeks]. Type 2 most commonly develops in individuals with sepsis following major intestinal resection. Patients require nutritional support for many weeks

or months, pending definitive surgery that may reverse dependency on PN. Type 3 is irreversible, for which long-term home parenteral nutrition (HPN) is required and is the focus of this article.

Intestinal failure in inflammatory bowel disease

Crohn's disease (CD) is most commonly associated with type 3 IF, but the overall incidence is low. The point prevalence of type 3 IF as a percentage of all causes in the United Kingdom in ulcerative colitis (UC) is 3% and 29% in CD^[3]. UC is much less commonly associated with type 3 IF because the small intestine is uninvolved, although IF can still occur through complications arising from delayed colectomy in immunocompromised patients, early re-operation, or mesenteric infarction after colectomy.

When IF does occur in patients with CD, it is usually due to one of three reasons: as a result of complications of surgery for intra-abdominal sepsis, extensive primary small bowel disease impairing nutrient absorption, or uncomplicated sequential resection leading to a shortened small bowel. The first is the principal cause of IF in CD^[4]. Following a first small bowel resection, the reported risk of IF in patients with CD at 5, 10, 15 and 20 years is 0.8%, 3.6%, 6.1% and 8.5% respectively^[5]. Predisposing factors to type 3 IF in CD include younger age at diagnosis and (at first operation) stricturing disease or family history of inflammatory bowel disease^[6]. In addition, the CD susceptibility gene nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is associated with IF in patients without CD^[7]. Whether this also applies to CD remains to be proven, despite established associations between NOD2 mutation and small bowel CD^[8].

MANAGEMENT OPTIONS FOR INTESTINAL FAILURE

Three options exist for the management of patients with type 3 IF: HPN, intestinal transplantation (ITx) and intestinal lengthening.

Home parenteral nutrition

HPN has formed the standard of care for managing patients with type 3 IF for several decades^[9,10]. Early regimes were complicated, but solutions have evolved to mixed-nutrient, stable, "single" or "bipartite" bags, meeting a patient's tailored nutritional requirements^[9,11]. These solutions can be delivered through long-term percutaneous intravenous catheters, specialised pumps and patients specially trained in self-administration, or specifically trained nursing staff. CD is the principal indication for HPN in the United Kingdom, although other disease aetiologies, such as cancer, form the principal indication in other countries^[12-15].

Intestinal transplantation

The first human small bowel transplant was in 1964

but, like many early organ transplants, the graft failed to survive^[16,17]. It was only after refinements in immunosuppression that ITx started to show promise, with the first successful small bowel (+ liver) transplant taking place in 1988, enabling the recipient to achieve nutritional autonomy^[18]. With further advances in immunosuppression and operative techniques, the number of transplants performed annually rose until 2005, since when it has remained stable^[19]. In the United Kingdom alone, the number of intestinal transplants has increased from single figures (2000-2008) to 14-22/year (2011-2013)^[20]. Currently, 100 ITxs are performed per year in adults, primarily in North America and Europe; 65% of ITx transplants between 2006 and 2011 were indicated for short bowel syndrome (SBS), of which 13% were in patients with CD^[19]. The number of transplants performed annually in other parts of the world, such as Asia and South America, is much lower, but gradually increasing^[19]. In total, 20 ITxs were reported to have been performed in Japan between 1996-2010, while Australia and India have both recently reported their first cases^[21-23]. To the best of our knowledge, there are no published reports of ITx in Africa.

At present, three types of graft transplants are performed: isolated intestinal, combined liver-intestinal and multivisceral transplantation. Combined liver-intestinal grafts include intestine, duodenum, liver and pancreas. Multivisceral grafts include intestine, stomach, duodenum, pancreas, possibly liver and colon, or other organs. At present, isolated small intestinal transplants are the commonest, although abdominal wall transplantation is increasingly combined with intestinal transplantation and provides a readily accessible marker for rejection^[19,24].

Currently, the choice between HPN and ITx as primary therapeutic options for patients with type 3 IF is principally driven by predicted survival outcome. Thus, HPN, with its superior long-term survival, remains the first-line management option for most patients with type 3 IF, with ITx being reserved primarily for those with HPN-associated complications and/or high risk of death from their underlying disease (Table 1). However, if transplant experience and survival continues to improve, other factors, such as patients' quality of life (QoL), will enter decision-making when balancing HPN against ITx.

Intestinal lengthening

Intestinal lengthening procedures involve the lengthways division of a dilated small intestine and subsequent end-to-end anastomosis ("Bianchi procedure") or sequential zig-zag stapling of a dilated small intestine (serial transverse enteroplasty or "STEP procedure")^[25-28]. Lengthening techniques were pioneered in children with SBS, and have rarely been performed in adults, although recent European and American experience suggests they may be a viable treatment option for SBS. However, as only one series has reported their use in 2 patients with CD, lengthening procedures will not be discussed further in this review^[29]. Instead, this review will focus on the choice between HPN and ITx in patients with type 3 IF.

Table 1 Intestinal transplantation indications^[53,57,92,94]

North American	European
Indications	Indication
Failure of home parenteral nutrition (HPN)	Irreversible, benign, chronic intestinal failure with no possibility of bowel rehabilitation associated with life threatening complications of HPN
Impending or overt liver failure	Individual case-by-case decision for all patients
Central venous thrombosis of ≥ 2 central veins	
Frequent and severe central venous catheter-related sepsis	Non-indications
Frequent episodes of severe dehydration despite intravenous fluids in addition to HPN	High risk of death due to underlying disease
High risk of death attributable to the underlying disease	Chronic dehydration
Intra-abdominal invasive desmoids tumour	Significantly impaired quality of life
Congenital mucosal disorders	
Ultra-short bowel syndrome	
Intestinal failure with high morbidity and low acceptance of HPN	
Need for frequent hospitalisation, narcotic addiction or inability to function	
Patient's unwillingness to accept long-term HPN	

PATIENT SURVIVAL

Survival on home parenteral nutrition

HPN series provide survival information, although series (Table 2) excluding cancer as a primary disease indication clearly have more relevance to IBD and ITx^[14,30-32]. Of the latter, one series (1986-2001; $n = 40$) reported 1-, 3- and 5 year probability survivals on HPN of 97%, 82% and 67%^[31]. Another study (1990-2006; $n = 268$) excluding malignancy, but only including patients with SBS, reported 1-, 5- and 10-year actuarial HPN survivals of 94%, 70% and 52%^[30]. However, as 46% regained nutritional independence, most within 1 year, and only 6% had CD, this study does not represent ITx candidates or most patients with CD needing intravenous nutritional support. Indeed another study (1979-2003; $n = 188$), including 7% with malignancy (4% active neoplasia; 3% desmoid), showed that patients with CD on HPN ($n = 60$) had a better 5-year probability survival than all other patients (87% *vs* 77%)^[33]. This is further supported by a review of case series, which reported the 10-year survival rate for patients with CD to be 88% in comparison to 62% for SBS due to other causes and 60% for pseudo-obstruction^[34]. Thus, in general, patients with CD have the best probability of surviving long-term on HPN, which may reflect their age or limited co-morbidity (compared, for example, with those with SBS from mesenteric infarction). However, not all patients with CD have similar chances of long-term survival, as generic series show

that having < 50 cm of remaining small bowel (RR = 7.7) or an end-enterostomy (RR = 6.2) are associated with worse survival^[35,36].

Survival following intestinal transplantation

Survival following ITx (Table 2) currently appears worse than for patients on HPN, with the American National Registry reporting 1-, 3- and 5 year survivals of 77%, 61% and 51% for all primary adult ITxs (1987-2009; $n = 687$)^[37]. The trouble with direct comparisons with HPN survival data is, however, simple: the patient populations differ, since only a minority on HPN and predominantly those with established complications from HPN would be considered to be transplant candidates. Series highlight that graft and patient survival have improved considerably since earlier transplants; the American National Registry demonstrates a rise in 1-year survival from 69% in 1998 to 79% in 2007^[38]. This improvement is particularly evident in centres performing larger case volumes; for example, 5-year survival in Pittsburgh improved from 40% in 1990-1994 to 68% in 2001-2003^[19,39].

There are few survival data specific to adults with CD, with only one multi-centre series (1987-2009; $n = 86$) reporting 1-, 3- and 5 year survivals of 79%, 53% and 43% in patients with CD as the primary cause of IF^[24]. As in other series, 5-year survival from more recent procedures (2001-2009) has increased (62% isolated ITx; 57% liver-ITx). Post-ITx survival in adults with CD therefore appears comparable to those of other diseases. As with all patients, negative predictors for post-ITx survival in CD include age > 40 years and hospitalization prior to ITx^[24,37]. Furthermore, although the presence of NOD2 mutations are associated with an increased risk of rejection, graft loss and death in all patients post-ITx, this effect is not specific to CD^[40].

HOME PARENTERAL NUTRITION COMPLICATIONS

Catheter-related complications

Complications (Table 3) including catheter-related blood stream infections (CRBSI) and central venous thrombosis (CVT) are a significant cause of morbidity in patients requiring HPN and form part of the indications for ITx (Table 1).

Reported rates of CRBSI vary from 0.1/1000-2.41/1000 catheter days^[41-43]. Centre practices may influence rates; for example, increased use of lipid infusions or catheter use for infusions other than PN, are associated with an increase in CRBSI^[44]. CD may also increase risk, with one series of patients with CD describing 57% of patients having at least one CRBSI within the 7.9 years follow-up, and another series comparing patients with and without CD, reporting an association between CD and infections, attributed to immunosuppression and/or genetic immunodeficiency^[5,45]. Most CRBSI are bacterial (some are fungal) and remain a major concern, with between 4.5% and 16% of all HPN deaths attributed to CRBSI^[35,36,46]. It

Table 2 Comparison of patient survival

		1-yr	3-yr	5-yr	10-yr
Home parenteral nutrition	Series of 40 patients excluding malignancy (1986-2001) ^[31]	97%		82%	67%
	Series of 268 patients with SBS and excluding malignancy (1990-2006) ^[30]	94%		70%	52%
	Patients with Crohn's disease (CD) extracted from multiple series ^[34]				88%
	Series of 60 patients with CD (1979-2003) ^[33]			87%	
	Series of 453 patients (1990-2008) ^[39]	85%		61%	42%
Intestinal transplantation	Series of 687 patients (1987-2009) ^[37]	77%	61%	51%	
	Series of 86 patients with CD (1987-2009) ^[24]	79%	53%	43%	

is however clear that meticulous patient and carer training can achieve the very low CRBSI rates reported by some centres^[41,43].

Catheter-related CVT is less common than CRBSI, with recent series reporting 0.06-0.16 episodes of CVT/1000 d PN^[33,43,47]. Nevertheless, at one centre, the mean number of thrombosed central veins per patient at the point of ITx assessment, was 1.495^[48]. In an older series of patients with CD on HPN (1987-2009; *n* = 86), 50% were reported to have exhausted vascular access^[24]. This is clearly a concern for patients facing ITx, where vascular access is of paramount importance. CVT remains a prime consideration when determining an individual's referral for ITx assessment^[48,49].

Intestinal failure-associated liver disease

Intestinal failure-associated liver disease (IFALD) in children can be graded as early/mild, established/moderate and late/severe based on biochemical, histological and clinical parameters^[50]. With late disease, clinical and radiological signs of liver failure are accompanied by extensive hepatic fibrosis. IFALD incidence varies between centres, with one series reporting no patient with a bilirubin > 50, no decompensated liver disease or IFALD-related deaths in 107 HPN patients over a median of 40 mo (range: 4-252 mo)^[51]. Meanwhile, at the other extreme, another series of 90 HPN patients (median HPN duration 49 mo, range: 6-198 mo) reported complicated liver disease (as defined by bilirubin > 60, decompensation or fibrosis/cirrhosis on biopsy) in 50% of patients at 6 years; there were 6 IFALD-related deaths in the latter series^[52]. IFALD is associated with increased risk of death on HPN, but in light of its variable frequency, mortality also differs between centres (0%-22% of deaths)^[51-53]. These differences may reflect differing HPN management decisions, leading to variable exposure to risk factors, such as excess calories (especially lipids), underlying diseases (*e.g.*, bacterial overgrowth in CD) and recurrent episodes of sep-

Table 3 Potential complications of home parenteral nutrition and intestinal transplantation

Home parenteral nutrition	Intestinal transplantation
Catheter-related blood stream infection	Allograft rejection
Catheter-related central venous thrombosis	Infection
Intestinal failure-associated liver disease	Graft <i>vs</i> host disease
	Post-transplant lymphoproliferative disease
	Renal failure
	Disease recurrence

sis^[51,52,54,55]. Careful PN lipid formulation certainly seems to have a role in prevention and treatment^[51,52,56].

Given its association with death, IFALD is an indication for ITx in most countries^[53,57]. Patients with impending (raised bilirubin, progressive thrombocytopenia, or splenomegaly) or overt liver failure (portal hypertension, hepatosplenomegaly, fibrosis, or cirrhosis) should be considered for ITx^[53,57]. Traditionally, stratification of waiting times for liver-ITx was influenced by the model for end-stage liver disease (MELD), and paediatric version, pediatric end-stage liver disease. However, deaths on the waiting list in those awaiting combined liver-ITx were 8 times higher compared to liver alone^[38]. As a result these scores were adjusted to incorporate a sliding scale of 10% mortality at 3 mo. Over time this has reduced time waiting for a transplant, increased the number of liver-ITx and narrowed the gap between the two groups in both paediatric and adult populations^[58]. In addition, the MELD score and C-reactive protein have been shown to be independent predictors of survival in IF and may also be considered as reasons for early ITx assessment^[59]. Future areas for research include algorithms that may predict risk of developing IFALD. In reality, whilst liver biopsy remains the gold standard for assessing hepatic disease, non-invasive markers, such as Fibroscan[®], are gathering popularity. Rigorous data on its predictive value are needed. If a Fibroscan[®] score equated to a level of hepatic injury that in turn predicted the risk of IFALD, then there would be a strong argument for tailoring lipid exposure and total caloric intake to reduce this risk. As yet there is insufficient evidence to justify its use as a monitoring tool for patients on HPN.

Assessment tools

The Cambridge-Miami (CaMi) assessment tool has undergone preliminary validation to predict ITx outcome according to an individual's venous access and co-morbidity^[48,49]. It was developed as a pre-operative scoring system to help quantify the likelihood of survival after isolated ITx or as a composite graft, to help assess patients. The score combines risk factors for early-, medium-, and long-term survival, including loss of venous access and impairment of organs or systems not corrected by transplantation, each scored 0-3. Initial validation examined the preoperative scores of 20 patients who had

received intestinal transplants either isolated or as part of a cluster graft, who had either been followed up postoperatively for at least 10 years, or died within 10 years and compared with their survivals. A CaMi score < 3 was associated with survival ≥ 3 years (12/12 patients) and > 3 with survival < 6 mo (4/4). It is simple, disease-specific and is undergoing prospective validation, but does not examine QoL.

INTESTINAL TRANSPLANTATION COMPLICATIONS

Post-ITx complications (Table 3) may result in graft failure or death. Graft failure leads to patients resuming HPN and the need to consider re-transplantation, which has a lower probability of success than the index transplant^[37]. Graft failure is common, with reasons including allograft rejection, graft-*vs* host disease (GVHD), infection, post-transplant lymphoproliferative disorder (PTLD), primary non-function, or technical complications^[39]. Most graft failure occurs within the first few years. The North American Registry reported graft failure rates at 0.5-, 1-, 3- and 5 years of 16%, 26%, 46% and 48%^[60]. Graft survival in CD (2001-2009; $n = 63$) at 1-, 3- and 5 years is reported to be 90%, 65% and 52% for isolated-intestinal grafts and 65%, 57% and 57% for liver-intestinal grafts^[24]. Notably, the reason why liver-ITx grafts in the latter series of CD patients fared worse than in patients with other primary disease remains unexplored.

Allograft rejection

Rejection occurs *via* an immune-mediated response, which may be acute (cellular or vascular) or chronic^[39]. Although the incidence of rejection has fallen with improvements to immunosuppressive regimes, it remains a common problem. While not all episodes of rejection result in graft loss, they are associated with substantial morbidity^[39]. Acute cellular rejection has been reported to occur in 50%-75% intestinal transplants (1990-2008; $n = 500$) varying, with immunosuppressive regime, while acute vascular rejection occurred in 6% of isolated intestinal grafts (1990-2008; $n = 215$), of which 92% responded to treatment with anti-lymphocyte therapy^[39]. Chronic rejection occurred in 15% of all grafts (1990-2008; $n = 500$), but as indicated above, liver-containing grafts showed a significantly better chance of avoiding rejection than liver-free grafts, presumably due to the transplanted liver's immune-protective properties^[39,61]. In patients with CD, acute rejection has been reported to be the commonest cause of graft failure in the first 3 mo (33%), while chronic rejection was the commonest cause between 1-5 years (28%)^[24].

Infection

Immunosuppression minimises rejection, but renders recipients vulnerable to environmental and donor infections, with resultant morbidity and mortality^[62]. Infec-

tions are the second commonest cause of graft failure, accounting for 11% failures in a general ITx series (1990-2008) and 18% (1987-2009) in a CD ITx series^[24,39].

In one study 100 infections were reported in 19 ITx recipients during a median 524 d (18 mo) follow-up, with 94% having at least one bacterial infection^[63]. A larger study (1994-2001; $n = 124$) reported 2.6 episodes/patient^[64]. Bacterial infections are commonest, representing 61% of infections in one series, with septicæmia in 15%^[64]. However, the risk of fatal bacterial infections has declined following changes to immunosuppression regimes^[39].

Viral infections, particularly cytomegalovirus (CMV) and Epstein Barr virus (EBV), are potent causes of post-ITx morbidity, but the risk is declining, with altered immunosuppression regimes, viral monitoring and prophylaxis, and the matching of CMV donor to recipient status^[39,65,66]. In a recent series, (2001-2008; $n = 322$) 11% of ITx recipients were infected but none died^[39].

Graft vs host disease

ITx recipients are at high risk of developing GVHD, with one centre (1994-2007; $n = 241$) reporting GVHD in 9% of recipients, with children being at greatest risk (12.4% *vs* 4.6% adults, $P = 0.05$)^[67]. Isolated ITx have a lower risk than multivisceral grafts (4.4% *vs* 13.2%, $P = 0.05$). When GVHD does occur, it has a high mortality: in one series (1990-2008; $n = 500$), 18% of those affected died^[39]. There are no data to show whether ITx recipients with CD as the primary disease have an altered incidence of GVHD.

Post-transplant lymphoproliferative disease

Immunosuppression increases the risk of malignancy (8.7 times higher than general population), with the commonest being PTLD, which is associated with 1% of graft failures (2001-2008) and a high mortality (29% affected died; 1990-1995)^[39,68,69]. Recipients may be affected early or late following ITx, as shown by rates of 2.5%, 5.3%, 7.2%, 8.2% and 10.2% at 0.5-, 1-, 2-, 3- and 5 years post-ITx in one series (2005-2009)^[60]. Risk factors include EBV infection, which is present in 97%, immunosuppression and splenectomy^[39]. CD has not been investigated as a risk factor for PTLD.

Renal failure

Renal dysfunction is common in patients requiring HPN due to chronic dehydration from SBS and oxalate nephropathy, associated with jejuno-colonic anastomoses that are not uncommonly formed following CD resection. Although recurrent episodes of dehydration may be considered an indication for ITx, the actuarial incidence of significant renal dysfunction as a referral criterion for ITx (usually including multivisceral transplant) is uncommon^[53].

The risk of chronic renal failure is higher following ITx than in patients remaining on HPN^[70]. In the first year following ITx, 80% of adults experience an episode of acute kidney injury^[71]. Isolated small intestinal reci-

ents have a significant decline in renal function at 1 year, but multivisceral recipients do not, which may relate to their differing immunosuppressive regimes, since high dose tacrolimus is a risk factor^[71-73]. At one centre, 9% of surviving adult recipients required renal replacement therapy during a median follow-up of 7.6 years, with 50% attending for dialysis and 50% undergoing renal transplant^[74]. Furthermore, renal dysfunction at 1 year is a risk factor for mortality^[72]. Whether or not patients with CD undergoing ITx have an increased risk of renal impairment due to oxalate exposure or other factors remains unexplored.

Disease recurrence

Patients may view ITx as a cure for CD and, theoretically, donor graft genetics may reduce the risk of CD recurrence. However, case reports describe 2 patients, transplanted in 1994, who later developed clinical and histological recurrence (7 mo and 8 years post-ITx)^[75,76]. In another series, up to 19% of ITx survivors with initial CD had a recurrence suggested on routine histological assessment, but this did not affect graft function^[74]. Similarly, another small study reported asymptomatic CD recurrence in 50% (2/4) of patients, which was evident only on mucosal biopsy specimens (granulomatous enteritis)^[77]. Patients should therefore be advised that CD may reoccur in the grafted tissue, but that this may not manifest clinically, perhaps due to the effects of post-ITx immunosuppression.

QUALITY OF LIFE

Generic and disease-orientated tools exist for the assessment of QoL. Generic tools completed by patients on HPN and/or following ITx include the SF-36, Karnofsky performance score and QoL Inventory^[74,78-80]. The value of generic tools, including EQ5D (EuroQoL) which is used by National Institute of Clinical Excellence to calculate quality-adjusted life years, is that they are validated in many diseases, allowing comparisons with QoL in other chronic conditions, and in many languages^[81]. Their disadvantage is that they give little weight to disease-specific factors, such as a stoma or need for parenteral fluids. Disease-orientated tools have been developed, including both the Short Bowel Syndrome-Quality of Life Scale for patients with SBS and the HPN-QoL, for patients with IF on HPN^[82,83], which has been partially validated. An adapted version of the HPN-QoL has been used post-ITx^[84].

Quality of life on home parenteral nutrition vs intestinal transplantation

The SF-36 and an adapted version of HPN-QoL have been used to compare patients on HPN and following ITx. One study using the adapted HPN-QoL, found ITx recipients scored statistically better for ability to holiday/travel, fatigue, gastrointestinal symptoms, stoma management/bowel movements and global health status/quality of life and non-significantly better for eating ability^[84].

However, ITx recipients scored worse for sleeping pattern. Another study using SF-36, compared ITx recipients with patients stable on HPN and those with complicated IF on HPN, who were defined as those referred for ITx but who remained on HPN for whatever reason. Better QoL in ITx recipients and patients stable on HPN was reported than in those with complicated IF on HPN, suggesting that the benefit of ITx over HPN is limited to selected patients^[85]. This is to be expected, since patients on stable HPN not being considered for ITx cannot reasonably be compared to ITx. Another study, limited by low numbers from a single centre in the comparator group, compared QoL in those transplanted with those on stable HPN and found no difference between pre-ITx and stable HPN, but a significantly higher QoL score post-ITx^[78]. Since all these studies were small ($n = 55, 22$ and 59) and included patients who had undergone a variety of grafts for differing indications and at varying intervals, larger prospective assessments with disease specific tools are needed to confirm these findings, before QoL can be used to guide ITx decision-making. The optimal study would compare outcomes of those undergoing ITx for HPN failure compared to those with poor QoL at risk of HPN failure^[78]. No studies have examined QoL pre- and post-ITx in patients with IBD.

ECONOMIC CONSIDERATIONS

Both HPN and ITx impose financial burdens on the healthcare system and the patient. HPN cost estimates differ between countries and health services. In North America, HPN is estimated to cost \$64000/year^[86]. In the United Kingdom, HPN costs £30-40000/year, for 5 d/wk if self-caring, or £55-65000/year if requiring nursing support^[87]. ITx in the United Kingdom is estimated to cost £80000 in the first year, followed by £5000 annually. Thus, assuming no complications arise, ITx should be cost-effective after 2 years^[88]. Another European group drew similar conclusions when they reported an initial HPN fee of €9006, followed by €63000 annually, compared to €73000 initially for ITx followed by €13000 annually^[89].

HPN and ITx both affect an individual's economic situation. In some countries, patients are liable for a proportion of their healthcare cost, which places pressure on the patient to be in gainful employment. Assessment of employment status has been studied, but heterogeneity of the studies has produced variable data. For example, a recent review of QoL found that the employment rate after commencing HPN was 0%-52%^[90]. In contrast, in the last 500 transplants from Pittsburgh, 31% of their 151 adult patients were in employment or education^[39]. At a subsequent paper assessing long-term outcomes, of their surviving adult patients, 41 (35%) were in employment^[74]. The only comparative study between HPN and ITx was a cross-sectional study, where demographic data in a QoL study reported 56% (6% unemployed) of ITx recipients in part or full-time employment, compared to 30% (52%

unemployed) of patients on HPN ($P = 0.013$)^[84].

INDICATIONS FOR INTESTINAL TRANSPLANTATION

Decisions regarding the role of ITx *vs* HPN in type 3 IF necessarily consider many factors. Guidelines produced by the American Society of Transplantation (AST) (Table 1) are based on the premise that HPN still offers patients the best chance of long-term survival. Current guidelines therefore state that ITx should only be considered for patients with complications associated with HPN^[57]. These vary, ranging from life-threatening IFALD, recurrent CRBSI or limited venous access from CVC. Notwithstanding limited evidence of benefit, QoL can be included in the decision-making process. Thus, of these indications, HPN failure is the commonest (62%), followed by risk of death from underlying disease (26%) and high morbidity IF or low acceptance of HPN (12%)^[91]. More recent European guidelines suggest that indications for ITx should be restricted to complications associated with a higher mortality and do not support ITx for indications such as chronic dehydration or poor QoL^[92]. Further to evaluate the indications, Pironi and colleagues recently carried out a multi-centre, 5-year prospective follow-up of 545 European patients with type 3 IF, stable on HPN; patients were divided into two groups based on their candidacy for ITx according to AST criteria (Table 1). Within these groups, only those with desmoids or IFALD were associated with an increased risk of death on HPN, leading the authors to suggest that early referral for ITx should be mandatory for patients with these conditions. By contrast, patients with central venous catheter (CVC) complications or ultra-short bowel did not have an increased risk of death on HPN. Since there was no difference in survival in these groups whether they were transplanted or not, the authors concluded that CVC complications and ultra-short bowel be considered indications for ITx on a case-by-case basis. Notably, no patient who was considered to be an ITx candidate as a result of poor QoL or chronic dehydration actually died whilst remaining on HPN. The authors therefore concluded that these complications should not be considered an indication for ITx. Relatively few patients of the entire cohort underwent a transplant ($n = 22$), with a 5-year mortality rate of 54%. All deaths in transplanted patients were related to the transplant itself or to complications resulting from immunosuppression.

After this paper the European Society of Parenteral and Enteral Nutrition suggested that – at least in Europe – indications for ITx should be restricted (summarised in Table 1). This conclusion has been questioned by North American colleagues among others, who highlighted that the relatively poor survival rate of transplanted European patients, compared to 75% 5-year survival in a larger ($n = 182$) North American series over the same period^[93]. Indeed, it has been suggested that the poor European survival may relate to inadequate experience, since data

from the International Intestinal Transplant registry demonstrate improved graft survival in centres performing more cases^[19,93]. This trans-Atlantic debate remains unresolved, with Pironi and colleagues pointing out that some European ITx candidates (catheter complications and ultra-short bowel) had comparable survival figures on HPN and post-ITx to those of equivalent Pittsburgh ITx recipients^[93]. A key point when considering the risks and benefits of ITx *vs* HPN is that while ITx centre experience and/or outcomes may vary, the same is equally true of HPN experience and outcome. As earlier indicated, quality HPN outcomes such as the incidence of IFALD and catheter-related complications, vary appreciably between different HPN centres^[34,36,42,51,52]. Consequently, while ITx survival is likely to continue to improve and indications for ITx will shift as experience evolves, it is also essential that patients with type 3 IF are managed in expert IF centres with optimal HPN quality outcomes^[53].

CONCLUSION

Current management options for patients with irreversible IF secondary to IBD are HPN and ITx. For most patients, HPN has the more favourable survival and complication profile, but for selected patients, such as those with IFALD or specific catheter-related complications, ITx may offer better survival. As experience and outcomes in ITx improve, indications for ITx will no doubt widen. In the meantime, further work into tailoring the indications for ITx to individual patients will facilitate better selection. Since patients with CD have one of the better outcomes on HPN, the future use of tools such as CaMi, along with tailoring selection based on the predicted survival on HPN according to the primary disease aetiology, will facilitate patients' choice between ITx and HPN.

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Microscopic features of colorectal neoplasia in inflammatory bowel diseases

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Abstract

The risk of developing dysplasia leading to colorectal cancer (CRC) is increased in both ulcerative colitis and Crohn's disease. The prognosis of CRC may be poorer in patients with inflammatory bowel disease (IBD) than in those without IBD. Most CRCs, in general, develop from a dysplastic precursor lesion. The interpretation by the pathologist of the biopsy will guide decision making in clinical practice: colonoscopic surveillance or surgical management. This review summarizes features of dysplasia (or intraepithelial neoplasia) with macroscopic and microscopic characteristics. From an endoscopic (gross) point of view, dysplasia may be classified as flat or elevated (raised); from a histological point of view, dysplasia is separated into 3 distinct categories: negative for dysplasia, indefinite for dysplasia, and positive for dysplasia with low- or high-grade dysplasia. The morphologic criteria for dysplasia are based on a

combination of cytologic (nuclear and cytoplasmic) and architectural aberrations of the crypt epithelium. Immunohistochemical and molecular markers for dysplasia are reviewed and may help with dysplasia diagnosis, although diagnosis is essentially based on morphological criteria. The clinical, epidemiologic, and pathologic characteristics of IBD-related cancers are, in many aspects, different from those that occur sporadically in the general population. Herein, we summarize macroscopic and microscopic features of IBD-related colorectal carcinoma.

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Key words: Inflammatory bowel disease; Dysplasia; Colorectal cancer; Microscopic features

Core tip: The risk of developing dysplasia leading to colorectal cancer is increased in both ulcerative colitis and Crohn's disease. The biopsy interpretations will guide decision making in clinical practice: colonoscopic surveillance or surgical management. This review summarizes histological features of dysplasia and colorectal cancer in inflammatory bowel disease.

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INTRODUCTION

The most common types of inflammatory bowel disease (IBD) are ulcerative colitis (UC) and Crohn's disease (CD). The risk of colorectal cancer is increased in both UC^[1]

and CD^[2,3]. The prognosis of colorectal cancer (CRC) may be poorer in patients with IBD than in those without IBD^[4]. It is the pathologist's biopsy interpretations that guide the management of patients during surveillance^[5]. Pathologic interpretation of specimens for evaluation of dysplasia constitutes a critical step in endoscopic surveillance programs or surgery. Ultimately, it is the pathologist's interpretation of mucosal biopsy specimens that distinguishes high-risk from low-risk populations and triggers recommendations for either continued surveillance or surgery. Thus, an accurate diagnosis of dysplasia (or intraepithelial neoplasia) is the most important step in the surveillance process.

We review here the pathological characteristics of IBD-related colorectal cancer and dysplasia.

Dysplasia (intraepithelial neoplasia) in IBD

Most CRCs, in general, develop from a dysplastic precursor lesion. Patients with IBD develop dysplastic lesions that can be polypoid, flat, localized, or multifocal^[6]. Colorectal dysplasia may be defined as an unequivocal neoplastic alteration of the intestinal epithelium that remains restricted within the basement membrane within which it originated^[7]. It is synonymous with the term *intraepithelial neoplasia* adopted by the World Health Organization and Vienna nomenclature systems (Table 1) for gastrointestinal neoplasia^[5].

ULCERATIVE COLITIS

Macroscopic classification of dysplasia

From an endoscopic (gross) point of view, dysplasia may be classified as flat or elevated (raised)^[8-11]. Flat dysplasia refers to endoscopically undetectable lesions, whereas raised dysplasia refers to any type of endoscopically detectable lesion^[12].

Raised dysplasia: Endoscopically visible dysplastic raised lesions within an area affected by UC can be divided in adenoma-like and non-adenoma-like lesions on the basis of their macroscopic characteristics^[12]. Raised lesions with dysplasia in UC have been broadly separated into those that appear similar to non-IBD related sporadic adenomas, referred to as “adenoma like” and those which do not resemble adenomas: “non-adenoma-like (the former term “DALM”)^[13]. Adenoma-like RLDs represent well circumscribed, smooth or papillary, non-necrotic, sessile, or pedunculated polyps that are usually amenable to removal by routine endoscopic methods^[13,14]. Non-adenoma-like lesions include velvety patches, plaques, irregular bumps and nodules, wart-like thickenings, stricturing lesions, and broad-based masses^[9,15-17] and are not usually amenable to removal by colonoscopic polypectomy. Non-adenoma, and adenoma like RLDs are differentiated on the basis of their gross (endoscopic) appearance. Histologic comparisons of individual morphologic features in DALMs and adenoma-like dysplastic polyps have indicated that DALMs show increased ar-

Table 1 Vienna classification of gastrointestinal epithelial neoplasia

Category	
1	Negative for neoplasia/ dysplasia
2	Indefinite for neoplasia/ dysplasia
3	Non-invasive low-grade neoplasia (low-grade adenoma/ dysplasia)
4	Non-invasive high-grade neoplasia High-grade adenoma/ dysplasia Non-invasive carcinoma (carcinoma <i>in situ</i>) ¹ Suspicion of invasive carcinoma
5	Invasive neoplasia Intramucosal carcinoma ² Submucosal carcinoma or beyond

Reproduced from Schlemper *et al*^[94]. ¹Non-invasive indicates absence of evident invasion; ²Intramucosal indicates invasion into the lamina propria or muscularis mucosae.

chitectural disarray^[18], villous architecture, and inflammation^[19], but these criteria have not been evaluated longitudinally and have so far lacked the statistical power to guide the management of patients with raised dysplasia^[10,18,19].

Dysplastic polyps that are either encountered in non-diseased areas of the colorectum, for example, proximal to the transition zone in UC^[20], or have a non dysplastic pedicle^[19,21,22], are considered to be sporadic adenomas unrelated to the colitis and are managed accordingly. The endoscopic, histologic, and prognostic similarities between adenoma-like polyps in IBD and sporadic adenomatous polyps suggest that some, if not all, of the former are merely fortuitous adenomas, a conclusion that is also supported by limited molecular-based evidence^[23]. Follow-up studies after their endoscopic removal have reported no significant excess risk for the development of CRC^[24-28]. This favorable outlook is maintained even when the resected polyps contain HGD^[28,29]. Adenoma-like lesions can be adequately treated by polypectomy provided the lesion can be completely excised, shows the absence of dysplasia at the margins of the specimen, and there is no evidence of flat dysplasia elsewhere in the colon, either adjacent to, or distant from, the raised lesion^[12].

Kisiel *et al*^[30] showed that, while polypectomy may be safe for the management of adenomas occurring in most UC patients, the 5-years cumulative incidence of a combined endpoint (cancer or flat dysplasia) was 13%. Such patients should be followed closely.

Flat dysplasia: Flat dysplasia refers to dysplasia that is detected unexpectedly in random biopsies of mucosa without a corresponding macroscopic lesion, although occult dysplasia is a more suitable term considering that small or subtle raised lesions might easily go unnoticed in the inflammatory background of IBD^[31]. Retrospective endoscopic studies have suggested that most dysplastic lesions are in fact endoscopically visible. On the basis of

Table 2 Biopsy classification of dysplasia in inflammatory bowel disease

Negative for dysplasia	Positive for dysplasia
Normal mucosa	Low-grade dysplasia
Inactive (quiescent) colitis	High-grade dysplasia
Active colitis	

a review of random and targeted surveillance biopsies in 525 subjects with UC during a period of 15 years, Rutter *et al*^[9] reported that 85 of 110 (77.3%) biopsy specimens of dysplasia or cancer corresponded to macroscopically visible lesions, whereas 25 (22.7%) were invisible. Similarly, on the basis of a review of surveillance biopsies in 46 subjects with UC during a period of 10 years, Rubin *et al*^[32] reported that 38 of 65 dysplastic lesions (58.5%) and 8 of 10 cancers (80.0%) were visible as 23 polyps and masses, 1 stricture, and 22 mucosal irregularities. Only some of the dysplastic lesions described as flat by endoscopists correspond to expanded mucosa resembling diminutive adenomatous polyps, whereas most correspond to histologically flat mucosa in which the crypts have been colonized by dysplastic epithelium, without alteration of the overall mucosal architecture.

Microscopic classification

Currently, dysplasia is separated into 3 distinct categories: negative for dysplasia, indefinite for dysplasia, and positive for dysplasia (low or high grade) (Table 2)^[7]. While endeavouring to minimize disagreement in both terminology and interpretation, rates of agreement using this grading system are only fair among both expert and community pathologists^[33]. Crude rates of agreement among experts have ranged from 42% to 72%; kappa values, where there is a correction for chance agreement, have remained fair for both experts and community pathologists^[33-36]. Unfortunately, rates of agreement are lowest for the indefinite for dysplasia and low-grade dysplasia categories^[24,33]. Based on these data, the CCFA consensus guidelines and the United States Multisociety Task Force strongly recommend that a second examination of the biopsies should be performed by an independent pathology expert prior to definitive treatment^[21,37].

The morphologic criteria for dysplasia are based on a combination of cytologic (nuclear and cytoplasmic) and architectural aberrations of the crypt epithelium^[7,19,38]. Cytologic features that pathologists use to evaluate the presence or absence and degree of dysplasia include the nuclear/cytoplasmic (N/C) ratio of the cells; loss of cell polarity; an increase in the number and location of mitoses (typical and atypical); the degree of nuclear stratification within the epithelium; the degree of chromasia of the nuclei (an increase is referred to as “hyperchromasia”); the presence, size, and multiplicity of nucleoli; the size and regularity (or lack thereof) of the contour of nuclei; and the variation in the size and shape of nuclei between different cells (nuclear pleomorphism). Cytoplasmic characteristics include the degree of mucinous depletion;

the number, location, and shape (normal or dystrophic) of goblet cells; and the presence or absence of surface maturation, which is defined as the progressive acquisition of cytoplasmic mucin, a decrease in the size of nuclei, and the degree of stratification of the cells, from the crypt base to the mucosal surface. Architectural features that are important for the determination of dysplasia include villiform change of the epithelium and the presence or absence and degree of crypt budding, branching, and crowding; the latter is referred to commonly as a “back-to-back” glandular growth pattern. In addition, the contour of the crypts, the degree of irregularity, and the presence or absence of intraluminal bridges (“cribriforming”) are important architectural features that are used to evaluate dysplasia in IBD^[39].

Negative for dysplasia: “Negative for dysplasia” applies to epithelium that is regenerative in nature. In the presence of active inflammation, cryptitis, crypt abscesses, or ulceration, all of which are common in the active phase of IBD, the epithelium can undergo marked reactive changes that, in some circumstances, may mimic some of the “atypical” features of dysplasia. In general, nondysplastic (“reactive”) epithelium in IBD exhibits only mild or moderate cytologic atypia coupled with preservation of crypt architecture; however, a significant degree of atypia may be present in markedly reactive epithelium adjacent to ulcerated mucosa, an area in which the architecture of the crypts may be altered as well. One of the hallmarks of reactive crypts is a base-to-surface epithelial maturation gradient in which phenotypically immature, mitotically active, basal colonocytes differentiate into mature surface cells, featuring small, normochromatic nuclei, distinct absorptive and goblet cell phenotypes, and absent mitoses^[5]. Pathologists need to exercise caution when evaluating dysplasia in ulcerated mucosa, and these areas should be avoided by the endoscopist when obtaining mucosal biopsies. Given the subtle gradation of changes, the progressive acquisition of molecular mutations that occurs in the progression of dysplasia in IBD^[38,40,41], and the wide range of morphologic patterns of atypia that is related to epithelial regeneration and repair, regenerating epithelium, particularly in the setting of active inflammation or ulceration, may reveal a level of atypia that occasionally is difficult to distinguish from true dysplasia^[41]. In these situations, pathologists use the “indefinite for dysplasia” diagnostic category. In reality, this diagnostic category is used most often as a result of one of the following circumstances: the presence of technical (tangential sectioning) or staining issues that makes interpretation of cytologic or architectural features difficult, atypia related to inflammation or ulceration, or for the rare instances in which dysplasia-like changes are present only in the crypt bases. Naturally, the frequency of the use of this diagnostic category is directly proportional to the “comfort” level and experience of the reviewing pathologist, and is one of the reasons why it is highly recommended to confirm any potential diagnosis of dysplasia with at least

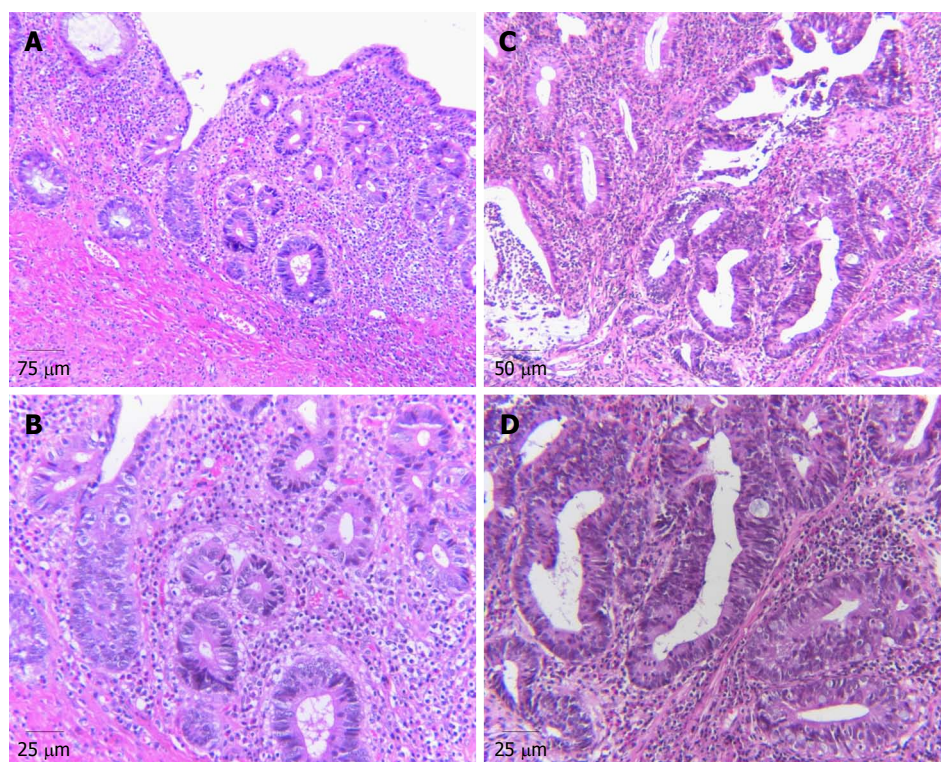


Figure 1 Microscopic features of dysplasia in ulcerative colitis. A: A minor degree of architectural aberration may occur in low-grade dysplasia (HE stain, $\times 100$); B: Low-grade dysplasia is characterized by epithelium that contains cells with significant nuclear hyperchromaticity, enlargement, and elongation. The cytoplasm is mucin depleted, and, as a result, is hypereosinophilic (HE stain, $\times 200$); C: The degree of architectural aberration is more prominent in high-grade dysplasia. Architectural aberrations, such as a complex crypt branching, or a back-to-back growth pattern that is characterized by dysplastic crypts that show little or no intervening lamina propria, also may be present (HE stain, $\times 400$); D: Full-thickness nuclear stratification, significant loss of cell polarity, nuclear pleomorphism are characteristic features of high-grade dysplasia (HE stain, $\times 400$).

one other experienced IBD pathologist before definitive treatment^[21,38].

Low-grade dysplasia: Low-grade dysplasia is characterized by epithelium that contains cells with significant nuclear hyperchromaticity, enlargement, and elongation; the last is referred to as “pencil-shaped” or “adenomatous” nuclei. Nuclei in low-grade dysplasia often show a clumped chromatin pattern, multiple nucleoli, or a single large nucleolus. Typically, the cytoplasm is mucin depleted, and, as a result, is hypereosinophilic. A decrease in the number of goblet cells and unusually oriented goblet cells, referred to as “dystrophic” goblet cells, may also be observed (Figure 1B). Dysplastic cells are usually organized in a stratified manner, but in general, the nuclei are limited to the basal half of the cell cytoplasm, without full thickness stratification. Mitotic figures may be prominent, but there are only usually a few atypical mitotic figures. Most importantly, dysplastic epithelium usually does not show surface maturation, except in rare circumstances. A minor degree of architectural aberration may occur in low-grade dysplasia (Figure 1A), but significant architectural aberration is normally diagnostic of high-grade dysplasia (Figure 1C)^[39].

High-grade dysplasia: With progression to high-grade dysplasia, the degree of cytologic or architectural aberration

is more prominent (Figure 1C). Cytologically, full-thickness nuclear stratification, significant loss of cell polarity, nuclear pleomorphism, and an increase in the number of normal appearing and atypical mitoses, often at the level of the surface epithelium, are characteristic features of high-grade dysplasia (Figure 1D). In some instances, high-grade nuclei are more round or oval in contour, and also show a higher N/C ratio. Architectural aberrations, such as a complex crypt budding or branching, or a back-to-back growth pattern that is characterized by dysplastic crypts that show little or no intervening lamina propria, may also be present (Figure 1C). Cystic change, villiform surface change, and cribriforming are also features of high-grade dysplasia^[42-44].

Immunohistochemical or molecular markers of dysplasia: Many studies have been published in an effort to help identify sensitive and specific immunohistochemical or molecular markers that may aid in the differentiation of dysplastic from reactive epithelium in IBD. p53 and Ki67 have been studied the most extensively. Most of the markers that were evaluated previously have been linked, in some capacity, to the development of cancer, and include those involved in control of cell proliferation (*e.g.*, Ki67, cyclin D1), intercellular adhesion (β -catenin, e-cadherin), DNA content, mucin or glycoprotein histochemistry, and tumor suppression (p53)^[40,45-59].

The *p53* gene shows an increase in the frequency of mutations in the dysplasia-carcinoma progression in IBD^[45-51]. *p53* is a common early mutation in the dysplasia-carcinoma sequence in IBD, and, as a result, many investigators have evaluated the role of *p53* in helping to differentiate reactive from dysplastic epithelium. For instance, in a study by Wong *et al.*^[45], a moderate degree of *p53* staining was detected in almost 50% of reactive cases, but strong *p53* staining was seen only in cases of true dysplasia. Unfortunately, although *p53* expression increases progressively from low- to high-grade dysplasia and carcinoma, some studies showed that the epithelium that is considered indefinite, or even negative, for dysplasia, may be *p53* positive; this diminishes its usefulness as a marker of true dysplasia^[42,45,47]. Furthermore, *p53* overexpression can be detected in a small proportion of cases that are considered morphologically negative for dysplasia^[45,47,49,50]. In addition, several studies in other tissues have revealed a high rate of false-positive staining in the absence of *p53* mutations, and a high frequency of false-negative staining as well^[60,61]. Nonspecific binding of *p53* to non-*p53* mutation-related antigens may also lead to false-positive results. Furthermore, *p53* results may vary substantially depending on the specific type of antibody used. For instance, some *p53* mutations result in the production of a protein that does not bind to some antibodies that are directed against the wild-type protein. Finally, there is no known antibody, or combination of antibodies, in use that can detect all *p53* mutations^[60]. For these reasons, *p53* immunostaining is not routinely used but can be helpful in rare cases to differentiate reactive from dysplastic epithelium in IBD.

Several studies showed that dysplasia expresses markers of cell proliferation at higher levels in the crypt, and in the surface epithelium, compared with biopsies that are considered negative for dysplasia^[45,46,58]. Unfortunately, there is much overlap between reactive epithelium and dysplasia in this regard, so evaluation of cell proliferation is not useful in individual cases to distinguish these lesions.

Recently, immunostaining for alpha-methylcyl-CoA racemase (AMACR), an antibody that is often used in the assessment of diagnostically difficult atypical, and potentially neoplastic, lesions of the prostate, was shown to have a high degree of specificity for detection of dysplasia in the GI tract, such as in Barrett's esophagus and IBD^[62]. In this recent study by Dorer and Odze^[62], AMACR was not expressed in any mucosal biopsy in UC that was considered negative for dysplasia; however, it was increased significantly in foci of low-grade dysplasia (96%), high-grade dysplasia (80%), and adenocarcinoma (71%) with a specificity for neoplasia of 100%. Thus, AMACR is a new, potentially useful immunohistochemical marker that pathologists may use in their arsenal when trying to differentiate reactive from dysplastic epithelium in IBD. More recently, Chen *et al.*^[63] showed that Chitinase 3-like-1 may contribute to the proliferation, migration and neoplastic progression of colonic epithelial cells

under inflammatory conditions and could be a useful biomarker for neoplastic changes in patients with IBD. More recently, Ludwig *et al.*^[64] show that PDCD4 nuclear expression may be usefully applied as ancillary marker in the histological assessment of IBD-associated dysplastic lesions.

Overall, dysplasia diagnosis is essentially based on morphological criteria.

Crohn disease

Less studied than in UC, dysplasia in CD occurs more often in areas close to, rather than distant from, the primary tumor mass. Dysplasia in CD is often multifocal^[65]. In a study by Sigel *et al.*^[66], dysplasia was found adjacent to carcinoma in 87% of cases and distant from carcinoma in 41% of cases. Microscopic features that are used for a diagnosis of dysplasia (or intraepithelial neoplasia) in CD are the same that those used in UC dysplasia.

Colorectal carcinoma in inflammatory bowel disease

The clinical, epidemiologic, and pathologic characteristics of IBD-related cancers are, in many aspects, different from those that occur sporadically in the general population. For instance, cancers that occur in IBD, and particularly UC, tend to be distributed more evenly throughout the length of colon, are more likely to be multiple in number and tend to be of higher histologic grade than with sporadic carcinomas^[67]. In some studies, up to 27% of IBD-related cancers are multiple in number^[68,69]. In addition, there is a higher prevalence of mucinous carcinomas in IBD^[67,70,71]. More recently, there has been a shift to a higher incidence of early-stage tumors (stage I - II) compared with IBD-related cancers from previous decades^[41,67,68]. Contemporary studies show that 50%-60% of newly diagnosed IBD-related cancers are stages I or II. Of course, this may be due to a combination of many factors, such as an increased level of awareness and early detection by colonoscopic surveillance. In one study, by Delaunoy *et al.*^[67], of 290 patients who had IBD (241 with UC and 49 with CD) and an equal number of age- and sex-matched patients who had sporadic colorectal cancer, UC-related carcinomas were diagnosed at a younger age and tended to be distributed more evenly in the colon, compared with sporadic tumors.

Macroscopic features

Pathologically, IBD-related tumors often grow in a more diffuse fashion than sporadic cancers, and may be more difficult to detect grossly because they may be raised only minimally above the level of the surrounding mucosa^[41,67]. The gross appearance of cancers in IBD is heterogeneous. They may be strictured, ulcerated, irregular, polypoid (pedunculated or sessile), or nodular or they may appear as an irregular plaque or bump^[41,71]. Some tumors may be entirely microscopic, without any grossly evident mucosal abnormality^[17,72]. A disproportionately higher percentage of cancers in IBD, including UC, occurs in strictured segments of colon^[73,74].

Microscopic features

Microscopically, most IBD-related carcinomas are adenocarcinomas. Mucinous carcinomas make up a high proportion, up to 50% in some studies^[40]. In addition, signet ring cell adenocarcinomas are 10 times more common in IBD than in the general population^[41,75]. Rarely, IBD-related adenocarcinomas may be extremely well differentiated and consist of widely separated, regularly arranged, bland-appearing glandular profiles that contain only mildly atypical unilayered neoplastic epithelium with low-grade cytologic atypia, and without desmoplasia^[41,76]. These tumors may arise from mucosa that shows little or no definite evidence of dysplasia. Other types of carcinomas, such as neuroendocrine carcinomas, mixed adenocarcinoma/squamous cell carcinoma, undifferentiated carcinoma, and even pure squamous cell carcinoma (particularly in the distal rectum and anal canal in CD), have been encountered in IBD; some occur with increased frequency^[40,41,73-83]. However, these tumors are rare and are often reported as single case reports or as small series.

Colorectal carcinoma in CD

Regarding CD cancers specifically, recent evidence suggests that the risk of cancer in CD is similar to that in UC, particularly for patients who have long-standing and extensive colonic disease^[38,40,84,85]. In contrast to UC, CD patients who develop cancer are often older in age, but are much younger than patients who develop sporadic colon cancer in the general population^[41,68,86]. Some early reports cited a higher left-sided predominance for cancers in CD compared with UC; however, several contemporary studies showed a more equal distribution of tumors in the colon in CD, similar to UC^[73,67,87]. Earlier studies may have been biased by the fact that cancers in CD often occur in and around the anal canal related to fissures or fistulas^[86-88]. As a result, there is an increase in the incidence of pure squamous cell carcinomas in CD compared with UC. Although most cancers in CD are believed to occur within inflamed portions of intestine^[40,89,90], in some studies, up to 42% of patients who had CD developed tumors in areas of mucosa devoid of endoscopic or pathologic evidence of inflammation^[73,86-88,91]; however, this may be due to treatment effect^[92]. Finally, surgically excluded segments of colon or small bowel also are considered particularly prone to the development of carcinoma in CD, but this is postulated to be related to the fact that excluded segments of inflamed bowel remain at risk for carcinogenesis for longer periods of time over the course of the patient's life^[40,41,67,87,93]. Because surgical procedures that result in preservation of inflamed but excluded segments of bowel are performed only infrequently in patients who have CD, cancers in surgically excluded segments of bowel are now uncommon.

ing carcinoma is related to the extent of the patient's disease (pancolitis *vs* left-sided disease), duration of disease, and level of activity. In IBD there is abundant evidence to support the theory that cancer develops through an inflammation-dysplasia-carcinoma sequence. Several molecular events involved in the chronic active inflammatory process contribute to multistage progression of carcinoma development. Morphologic identification of dysplasia in IBD is the best and most reliable marker of an increased risk for malignancy. Future advances in, for example, stool DNA assays or the use of confocal endomicroscopy or endoscopic ultrasound may help in the identification of high risk patients and the assessment of dysplastic lesion^[11].

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CONCLUSION

Patients with IBD, including UC and CD, are at increased risk of developing colorectal cancer. The risk of develop-

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Venous thromboembolism in patients with inflammatory bowel disease: Focus on prevention and treatment

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Abstract

Inflammatory bowel disease (IBD) patients have an increased risk of venous thromboembolism (VTE), which represents a significant cause of morbidity and mortality. The most common sites of VTE in IBD patients are the deep veins of the legs and pulmonary system, followed by the portal and mesenteric veins. However, other sites may also be involved, such as the cerebrovascular and retinal veins. The aetiology of VTE is multifactorial, including both inherited and acquired risk factors that, when simultaneously present, multiply the risk to the patient. VTE prevention involves correcting modifiable risk factors, such as disease activity, vitamin deficiency, dehydration and prolonged immobilisation. The role of mechanical and pharmacological prophylaxis against VTE using anticoagulants is also crucial. However, although guidelines recommend thromboprophylaxis for IBD patients, this method is still poorly implemented because of concerns about its safety and a lack of awareness of the magnitude of thrombotic

risk in these patients. Further efforts are required to increase the rate of pharmacological prevention of VTE in IBD patients to avoid preventable morbidity and mortality.

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Key words: Inflammatory bowel disease; Venous thromboembolism; Thromboembolic prophylaxis; Anticoagulants; Unfractionated heparin; Low molecular weight heparin

Core tip: Inflammatory bowel diseases (IBD) patients have an increased risk of venous thromboembolism (VTE) that represents a significant cause of morbidity and mortality. The prevention of VTE involves the correction of modifiable risk factors, such as: disease activity, vitamin deficiency, dehydration and prolonged immobilization. Essential is also the role of mechanical and pharmacological prophylaxis. However, thromboprophylaxis in IBD patients, although guideline-recommended is still poorly implemented because of concerns about its safety and, over all, the lack of awareness of the magnitude of thrombotic risk in these patients. Further efforts are required to increase the rate of pharmacological prevention of VTE in IBD so to avoid some preventable morbidity and mortality.

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INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's dis-

ease (CD) and ulcerative colitis (UC), are chronic disorders that predominantly affect the bowel; however, IBD can also be associated with numerous extraintestinal complications. Among these complications, venous thromboembolism (VTE) is particularly important, due to both its high prevalence and its significant morbidity and mortality^[1-9]. However, despite extensive evidence supporting the association between IBD and VTE, among physicians, there is still a lack of recognition of this risk, with dangerous consequences for patients^[8,10]. Thus, the aim of this review is to summarise the most recent evidence regarding the prevention and treatment of VTE in IBD patients in light of the newest epidemiological data on this feared association.

EPIDEMIOLOGY AND CLINICAL FEATURES OF VTE IN IBD PATIENTS

Epidemiological data

IBD patients have a 2- to 3-fold increased risk of developing deep venous thrombosis (DVT) and pulmonary embolism (PE) compared with the general population^[1,3-10]. In their population-based cohort study, Bernstein *et al*^[7] found an incidence rate of DVT of 30.7 per 10000 person-years in IBD patients (30.0 for UC patients and 31.4 for CD patients) and 14.9 per 10000 person-years for PE in the entire IBD population (19.8 for UC and 10.3 for CD). The overall relative risk (RR) of VTE reported in this study was of 3.47 (95%CI: 2.94-4.09)^[7]. These findings were confirmed by a recent, large population-based study from Denmark reporting an incidence rate of VTE of 24 per 10000 person-years among IBD patients (24.4 for UC patients and 23.3 for CD) compared with an incidence rate of 13.4 per 10000 person-years in a non-IBD cohort matched for age and gender^[4]. Although the incidence of VTE increases with age, the highest RR for VTE was observed among patients younger than 40 years of age^[3-7], whereas no significant differences linked to sex or the type of IBD were found^[1-10]. VTE occurred more frequently during phases of active disease and in patients with extended disease (pancolitis in UC patients and extensive colonic involvement in CD)^[3-6]. Recently, Grainge *et al*^[5] conducted an epidemiological study that aimed to quantify the risk of VTE during different activity phases of IBD. The researchers confirmed that IBD patients had the highest risk of VTE at the time of a flare (hazard ratio of 8.4 compared with controls), although an increased risk still persisted during disease quiescence (hazard ratio of 2.1 compared with controls). These findings support the hypothesis of a procoagulant tendency in IBD patients^[2,11]. Indeed, Miehsler *et al*^[3] demonstrated that VTE is a specific feature of IBD because neither rheumatoid arthritis, another chronic inflammatory disease, nor celiac disease, another chronic bowel disease, was accompanied by an increased risk of VTE compared with controls. Furthermore, these data were confirmed by the finding that among 17 chronic illnesses that were evaluated using the

United Kingdom primary care General Practice Research Database, only cancer and heart failure carried a greater risk of VTE than IBD did^[12]. Interestingly, another study reported that the RR of VTE in pregnant females with IBD was even greater than in pregnant females without IBD with an OR of 8.44 (95%CI: 3.71-19.20) for UC and 6.12 (95%CI: 2.91-12.9) for CD^[13]. Regarding the mortality rate in patients with IBD and VTE, the existing data indicate, significant 2.5-fold-increased odds of mortality associated with VTE-related hospitalisations compared with non-VTE-related hospitalisations^[8]. In addition, a revisit of a series of 98 IBD patients with VTE evaluated at the Mayo Clinic over a decade (1990-2000) reported a 22% mortality rate^[6], which is similar to the 18% mortality rate that was reported in a cohort of IBD patients with VTE two decades earlier at the same institution^[9].

VTE location

VTE occurs primarily in the deep veins of the legs and in the pulmonary system and, less frequently, in the cerebrovascular system, portal vein, retinal vein, and mesenteric veins (Figure 1)^[14-17]. Recently, a cohort study aimed to determine the location and clinical features of the first VTE in IBD patients and confirmed this finding^[18]. Of 157 IBD patients with a history of VTE, 142 (90.4%) had DVT and/or PE, whereas 15 (9.6%) had cerebral, portal, mesenteric, splenic or internal jugular vein thrombosis^[18].

Risk factors for VTE

Although a detailed exploration of risk factors is beyond the scope of this review, we must remember that VTE in patients with IBD is a multifactorial event that involves both hereditary and acquired factors that can coexist, thereby multiplying the individual prothrombotic risk^[2,11,19]. The main modifiable acquired risk factors for VTE in IBD patients are reported in Table 1. Several are intuitively more frequent in IBD patients compared with the general population, such as dehydration, indwelling catheters, prolonged immobilisation, hyperhomocysteinaemia, surgical interventions, and active disease with an “inflammatory burden”. The mutual interactions between inflammation and coagulation have been extensively studied, and IBD represents a paradigmatic model for this complex interplay^[20,21]. Indeed, in IBD, several mechanisms triggered by active inflammation are involved in moving the coagulative balance towards a prothrombotic state, including (1) increased plasmatic levels of recognised risk factors for thrombosis, several of which are also considered to be acute-phase reactants, and decreased levels of natural anticoagulants; (2) reduced fibrinolytic activity; (3) endothelial abnormalities that are mainly represented by the downregulation of the anticoagulant thrombomodulin and endothelial protein C receptor, which in turn affects the conversion of protein C into its activated form; and (4) abnormalities of platelets, such as thrombocytosis and increased activation and aggregation^[11]. Concerning the inherited risk factors for VTE, the most common factors are: factor V Leiden

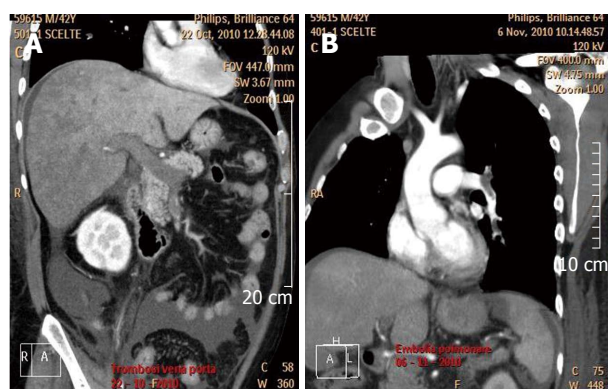


Figure 1 Computed tomography scan showing portal vein thrombosis (A) and a pulmonary embolism (B) in a patient with active ulcerative colitis.

mutation, G20210A mutation of the prothrombin gene, and homozygous C677T mutation in the methylenetetrahydrofolate reductase gene. However, no increased prevalence of these genetic prothrombotic factors has been found in IBD patients with or without VTE^[19]. Therefore, patients with IBD face both the VTE risk factors (acquired and inherited) identified in the general population and those specific to IBD, and the prevention and control of those risk factors are of paramount importance for thromboprophylaxis in this patient population.

PROPHYLAXIS AGAINST VTE IN IBD PATIENTS

Non-pharmacological prophylaxis

As described above, VTE in IBD is a multifactorial process in which acquired risk factors seem to play the most important role; therefore, these factors' prevention and/or treatment can lead to effective prophylaxis. Hydration, correction of deficiencies in vitamins (particularly in vitamins B6 and B12 and folate) that can reduce homocysteine levels^[22], graduated compression stockings or pneumatic devices, and early mobilisation after surgery should be always considered, especially in hospitalised IBD patients (Table 1). Additionally, even in the absence of direct evidence, it might be expected that the control of disease activity could decrease the risk of VTE by reducing the already-mentioned procoagulant factors that are closely associated with active inflammation. Furthermore, many drugs used for IBD treatment have shown anti-inflammatory activity and an anticoagulant effects. Indeed, mesalamine can reduce platelet activation^[23], azathioprine and 6-mercaptopurine inhibit platelet aggregation *in vitro*^[24], and infliximab normalises haemostatic parameters and reduces the amount of circulating microparticles and the levels of prothrombotic sCD40L in CD patients^[25,26].

Pharmacological prophylaxis

Prophylactic anticoagulation in IBD patients is recommended by several practice guidelines for conditions associated with a higher risk of VTE, particularly in hos-

Table 1 Acquired risk factors for venous thromboembolism in inflammatory bowel disease patients and modalities for their prevention and/or treatment

Risk factor	Prevention/treatment modality
Active disease ("inflammatory burden")	Effective anti-inflammatory treatment
Smoking	Programmes for smoking cessation
Oral contraceptive use	Advise alternative methods of contraception
Hyperhomocysteinaemia	Assess the presence of vitamin deficiency (vitamins B6 and B12 and folic acid) and correct if necessary
Dehydration	Provide adequate hydration
Prolonged immobilisation	Early mobilisation, especially after surgery; graduated compression stockings or pneumatic devices
Infections	Timely diagnosis and treatment of infections
Indwelling catheters	Limit the use of venous catheters; when possible, administer oral and enteral nutrition
Obesity	Encourage weight loss (diet, exercise)
Long-distance travel	Frequent ambulation, exercise, hydration

pitalised patients with active disease^[27-31]. Low molecular-weight heparin (LMWH) and unfractionated heparin (UH) are recommended for thromboprophylaxis in IBD patients. The recommendations for VTE prophylaxis in IBD patients that are included in the current guidelines are summarised in Table 2. Although no randomised controlled trials (RCTs) have specifically assessed the efficacy of anticoagulation for VTE prophylaxis in IBD patients, several RCTs have demonstrated that in acutely ill medical patients pharmacological prophylaxis significantly reduces the incidence of VTE^[32,33]. Only data from observational studies including IBD patients undergoing thromboprophylaxis with LMWH in the perioperative setting are available^[34,35]. Scarpa *et al.*^[34] collected data on 755 colorectal surgical procedures, 383 of which were performed in IBD patients. All patients had received 4000 IU/d LMWH from the day of operation through to the discharge. Six postoperative thromboembolic events occurred in this population, all in IBD patients; of these events, two occurred in CD patients (clinical DVT rate of 1.2%) and four occurred in UC patients (clinical DVT rate of 2.6%)^[34]. Similar data were reported by an Irish study in which the rates of postoperative VTE were evaluated both in 79 UC patients undergoing 180 major intra-abdominal surgeries and in 18 patients with familial adenomatous polyposis (FAP) undergoing 35 surgical operations of similar complexity^[35]. All patients were treated with standard perioperative VTE prophylaxis. Only three UC patients (1.7%) developed VTE, compared with no patients with FAP^[35]. Unfortunately, in both of these studies, a control group without prophylaxis was not included. Therefore, we can only hypothesise a benefit of LMWH prophylaxis because the VTE rates reported in a large cohort of hospitalised IBD patients in the United States were similar to those found in the above-mentioned studies. More specifically, Nguyen *et al.*^[8] extracting data from 73197 discharges for CD and 43645 discharges

Table 2 Published guidelines for the prevention of venous thromboembolism in inflammatory bowel disease patients

Scientific society (reference)	Recommendations	Type of population at risk
European Crohn's and Colitis Organisation (ECCO) ^[29]	Mechanical thromboprophylaxis and/or heparin administration (UH or LMWH)	UC
European Crohn's and Colitis Organisation (ECCO) ^[28]	Consider VTE prophylaxis (UH, LMWH, or fondaparinux) in all hospitalised patients	CD
British Society of Gastroenterology (BSG) ^[31]	Pharmacological VTE prophylaxis for hospitalised patients with severe UC	UC
American College of Gastroenterology (ACG) ^[30]	VTE prophylaxis with heparin for hospitalised patients with severe UC	UC
American College of Chest Physicians (ACCP) ^[27]	Mechanical thromboprophylaxis with GCS or IPC; anticoagulant thromboprophylaxis with LMWH, UH or fondaparinux when bleeding risk decreases	Acutely ill hospitalised medical patients at increased risk of thrombosis who are bleeding or at high risk of bleeding

GCS: Graduated compression stockings; IPC: Intermittent pneumatic compression; CD: Crohn's disease; UC: Ulcerative colitis; VTE: Venous thromboembolism; LMWH: Low molecular-weight heparin; UH: Unfractionated heparin.

for UC and found that the crude rates of VTE were 21 per 1000 hospitalisations for UC patients and 13.9 per 1000 hospitalisations for CD patients. However, we should note that in the population analysed by Nguyen *et al.*^[8] only 18% and 11% of CD and UC patients, respectively, underwent bowel surgery during their hospitalisations and that abdominal surgery is a strong predictor of developing VTE. In conclusion, available evidence on the efficacy of thromboprophylaxis in IBD is still scarce, and RCTs that aim to ascertain this issue are warranted.

Issues associated to the adherence to the pharmacological prophylaxis

Another key topic is the low rate of VTE pharmacologic prophylaxis in hospitalised IBD patients, and, particularly in those admitted for medical services compared with those admitted for surgery, despite the recommendations provided by the guidelines^[36,37]. The inadequate use of anticoagulants for VTE prophylaxis in IBD is mainly related to two factors: (1) gastroenterologists' lack of awareness of both the increased risk of VTE in IBD patients and the guideline-recommended use of pharmacological prophylaxis in hospitalised IBD patients^[38]; and (2) concerns about the safety of anticoagulant drugs in patients with active IBD^[36,39]. However, a recent retrospective study of 974 IBD inpatients with a reported rate of pharmacological prophylaxis of 80% at admission showed that the rates of major and minor bleeding were similar for patients who received VTE prophylaxis and those who did not^[36]. Moreover, VTE prophylaxis was not associated with major postoperative bleeding^[36]. Indirect evidence of the safety of anticoagulation in IBD patients during an active flare also comes from certain clinical trials in which UH or LMWH was used to treat UC^[40-42]. A meta-analysis of eight clinical trials showed that few serious adverse events were observed in patients treated with UH or LMWH compared with controls, with no significant difference in any trial^[41]. In particular, only in one study, three patients with moderate-to-severe UC included in the heparin group were withdrawn from the study because of worsening of rectal bleeding^[42]. One of these patients required urgent colectomy. Additionally, in

the control group, one patient developed toxic megacolon and underwent urgent surgery. The remaining seven clinical trials showed no bleeding-related adverse events in their heparin groups^[41]. All of this evidence confirms that the prophylactic use of anticoagulants in hospitalised IBD patients with acute disease is safe, despite the presence of bleeding at admission. Another unresolved issue is whether thromboprophylaxis should be extended to all ambulatory patients with disease exacerbation or only to a subgroup considered to be at a higher risk of VTE. As previously reported, the highest risk of VTE in IBD patients is during phases of active disease^[3-5]; however, in the context of active disease, Grainge *et al.*^[5] found that the RR was higher during non-hospitalised periods than during hospitalised periods (hazard ratio of 18.8 *vs* 3.2). These data suggest that hospitalisation should not be considered as the only discriminant factor for thromboprophylaxis and that anticoagulation could also be extended to a subgroup of ambulatory patients with active disease and other significant risk factors for VTE. However, this finding should be interpreted with caution because the absolute risk of VTE is much more informative than the RR, although not always known for an individual patient. In fact, in the same study, the absolute risk of VTE of a patient hospitalised for an IBD flare was nearly six times higher than the absolute risk during an ambulatory flare (37.5 per 1000 person-years *vs* 6.4 per 1000 person-years)^[5]. Thus, for each patient with active IBD, the absolute risk of VTE should be carefully assessed, including the personal and family histories of VTE, the presence of cardiovascular or respiratory diseases, obesity, information on the use of oral contraceptives and smoking status, the presence of genetic prothrombotic risk factors, reduced mobility, and the presence of venous catheters^[43]. Additionally, as previously reported, also disease features could help in assessing the individual prothrombotic risk^[3-10]. Lastly, it is well known that surgery represents a major risk factor for VTE, particularly in patients with IBD^[8], and thromboprophylaxis is universally performed during the perioperative period. A recent retrospective review of patient data obtained from the American College of Surgeons National Surgical Quality

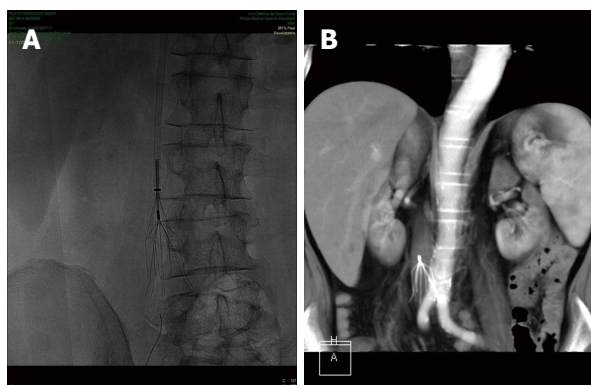


Figure 2 Plain abdominal Rx (A) and computed tomography scan (B) showing an inferior vena cava filter placed for the prevention of recurrent pulmonary embolism in a patient with Crohn's disease and deep venous thrombosis.

Improvement Program aimed to identify modifiable risk factors for short-term (30-d) postoperative VTE^[44]. The study reported that the following actions can potentially reduce the incidence of VTE in the surgical setting: correcting preoperative coagulopathy and/or anaemia, improving nutritional status, reducing steroid use, operating early to avoid emergency surgery, and limiting anaesthesia time^[44].

TREATMENT OF VTE IN IBD PATIENTS

The treatment of VTE in patients affected by IBD is the same as the treatment for subjects without IBD^[28,29]. If there is no haemodynamically significant bleeding or an indication of thrombolysis, LMWH is the ideal treatment. LMWH is usually switched to an oral vitamin K antagonist (*i.e.*, warfarin). The duration of therapy with anticoagulants is not well established because the possibility of VTE recurrence in IBD patients should be balanced with the bleeding risk caused by anticoagulant use. In fact, a recent study showed that IBD patients who experience their first episode of unprovoked VTE have a 33% risk of a second episode of VTE within 5 years, with a risk of recurrence that is 2.5-fold higher than that of non-IBD patients after an initial episode of unprovoked VTE^[45]. Nguyen and Bernstein^[46] conducted a decision analysis study to compare the costs and effectiveness of time-limited anticoagulation (for 6 mo) and extended anticoagulation for the management of VTE in IBD. The study found that among IBD patients who have had unprovoked VTE, the benefits of long-term coagulation in reducing recurrent VTE outweigh the risks of the associated bleeding. In particular, extended anticoagulation may be more appropriate for patients who developed VTE in the absence of active disease or other transient provoking factors^[46]. In the general population, local thrombolytic therapy is indicated for massive thrombosis and for life-threatening VTE, and several cases of successful catheter-directed thrombolytic treatment in IBD patients have been reported^[47]. Additionally, the placement of inferior vena cava (IVC) filters is indicated in cases of floating

thrombi in the deep veins of the legs and recurrent PE despite anticoagulant therapy and in cases with a high risk of bleeding (Figure 2)^[48].

CONCLUSION

IBD patients have a risk of VTE that is 2- to 3-fold greater than that of the general population. This risk is higher during disease flares, both for inpatients and outpatients. However, during hospitalisation, multiple prothrombotic risk factors other than active disease act synergistically, multiplying the absolute risk of VTE. Because VTE has significant morbidity and mortality, its prevention is mandatory. VTE prevention involves correcting modifiable risk factors and administering pharmacological prophylaxis. However, although guidelines recommend thromboprophylaxis for IBD patients, it is still poorly implemented because of concerns about its safety and a lack of awareness of the magnitude of thrombotic risk in these patients. Therefore, further efforts are required to increase the rate of pharmacological prevention of VTE in IBD patients to avoid preventable morbidity and mortality.

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Multipotent role of platelets in inflammatory bowel diseases: A clinical approach

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Abstract

There is evidence that inflammatory bowel diseases (IBD) combine both inflammation and coagulation in their pathogenesis and clinical manifestations. Although platelets (PLT) are well known for their role in hemostasis, there are a rising number of studies supporting their considerable role as inflammatory amplifiers in chronic inflammatory conditions. IBD are associated with several alterations of PLT, including number, shape, and function, and these abnormalities are mainly attributed to the highly activated state of circulating PLT in IBD patients. When PLT activate, they increase in size, release a great variety of bio-active inflammatory and procoagulant molecules/particles, and express a variety of inflammatory receptors. These inflammatory products may represent a part of the missing link between coagulation and inflammation, and can be considered as possible IBD pathogenesis instigators. In clinical practice, thrombocytosis is associated both with disease activity and iron deficiency anemia. Controlling inflammation and iron replacement in anemic patients

usually leads to a normalization of PLT count. The aim of this review is to update the role of PLT in IBD and present recent data revealing the possible therapeutic implications of anti-PLT agents in future IBD remedies.

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Key words: Anemia; Crohn's disease; Platelets; Thrombocytosis; Ulcerative colitis

Core tip: Many platelets (PLT) changes have been described in IBD, including morphological alterations (mean PLT volume, PLT distribution width, plateletcrit, and augmented granular content), count increase, microparticles release, over-excretion of granular content, and increased formation of PLT-PLT and PLT-leukocyte aggregates, which are all linked to PLT activation induced by inflammatory agonists. In this review article, we present the multipotent role of PLT in human biological paths and emphasize on how PLT participate in the chronic intestinal inflammation process in IBD.

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INTRODUCTION

Inflammatory bowel diseases (IBD), namely Crohn's disease (CD) and ulcerative colitis (UC), are disorders that primarily affect the gastrointestinal tract. The immune system, with its active components, dominates IBD pathogenesis, but many genetic and environmental factors have been also implicated. A growing number of

studies are highlighting the importance of non-immune cells like endothelial, mesenchymal, and nerve cells, as well as platelets (PLT), as key players in the IBD inflammatory cascade^[1].

PLT dysfunction is considered as participating in IBD pathogenesis, although the existing evidence is rather weak. On the other hand, there is solid evidence supporting PLT having functions of potent proinflammatory cells in addition to their role in hemostasis. Several studies have shown that PLT constitute a crucial link between inflammation and coagulation in both UC and CD, creating a vicious circle in which participating parameters conserve and propagate each other^[2].

Many PLT changes have been described in IBD, including morphological alterations [mean PLT volume (MPV), PLT distribution width (PDW), plateletcrit (PCT), augmented granular content], count increase, microparticles (MPs) release, over excretion of granular content, and increased formation of PLT-PLT and PLT-leukocyte aggregates (PLA), which are all linked to PLT activation induced by inflammatory agonists (Table 1). In the following sections, we will present the multipotent role of PLT in human biological paths and emphasize how PLT participate in the chronic intestinal inflammation process in IBD.

PLEIOTROPIC FUNCTION OF PLT

PLT are small anuclear fragments (1-6 μm) derived from bone marrow megakaryocytes, with a 5-9 d lifespan in humans. Their primary role is hemostatic; surveying endothelial barrier consistence and interfering when vessel integrity is threatened^[3]. A significant decrease of PLT ($< 20000/\text{mm}^3$) in septic models resulted in the disruption of the endothelial barrier in clinical studies^[4]. Collagen from the exposed subendothelial layer at the injured vessel site binds to plasma von Willebrand factor and recruits circulating PLT to form a glycoprotein (GP) Ib-IX-V complex. PLT adhesion to the site of injury initiates a cascade of signaling transduction through GP VI and integrin family surface receptors. PLT become activated and transform into high affinity platforms which are suitable for participating in inflammatory reactions, ligand binding, and clot formation promotion^[5]. In addition, PLT participate in wound repair and tissue regeneration by interacting with components of extracellular matrix and endothelium^[6,7].

It has been demonstrated that PLT present innate immunological properties. They express Toll-like receptors which can bind to lipopolysaccharides on the outer membrane of gram(-) bacteria^[8]. *In vitro* and *in vivo* studies have also demonstrated that PLT can internalize pathogens resistant to clearance such as *Staphylococcus aureus* or HIV virus, promoting further PLT activation changes^[9]. Moreover, PLT stimulate the formation of extracellular DNA nets by neutrophils that trap and kill gram(-) microbes, *via* the lipopolysaccharides - Toll-like receptor 4 interaction in septic models^[10,11].

Table 1 Platelet abnormalities in inflammatory bowel disease

Number and morphological changes	Loss of discoid shape Acquisition of pseudopodia Size increase Count increase (reactive thrombocytosis) Density increase Granular content augmentation MPV value decrease PDW value increase PCT value increase
Other abnormalities	
Overproduction and excretion of granular content products	P-selectin, β -TG, PF-4, fibrinogen, vWF, fibrinolytic inhibitors, coagulation, angiogenic and mitogenic factors
Increased incorporation of receptors in PLT membrane	CD40, P-selectin, GP53, GP IIb/IIIa, receptors for chemokines, cytokines and complement components
Overproduction of PLT-derived microparticles	
PLT-PLT aggregates formation	
Increased PLT-leukocytes formation	

β -TG: β -thromboglobulin; GP: Glycoprotein; IBD: Inflammatory bowel disease; MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet distribution width; PF-4: Platelet factor-4; PLT: Platelets; vWF: Von Willebrand factor.

PLT can also act as mediators between innate and adaptive immune systems. When activated at inflammatory sites, they excrete large amounts of pro-inflammatory substances located in their intracellular granules^[12], by which they crosstalk, recruit, and activate leukocytes, endothelial, and immune-like cells even at distant sites. A typical example of the remote PLT actions is the ability of PLT-derived CD40 ligand (CD40L) to activate dendritic cells in the injured tissue^[13] and to stimulate immunoglobulin production by B-cell compartment^[14].

PLT ability to interact with a large variety of cells is also implicated in the generation of vascular inflammation. Endothelium dysfunction triggers PLT activation processes and possibly renders PLT as the first in line to initiate atherosclerotic immune responses. Therefore production and release of PLT highly inflammatory cargo at the injured vessel wall induces and propagates the recruitment of leukocytes and the further construction of atherosclerotic lesions.

QUANTITATIVE AND QUALITATIVE PLATELET CHANGES IN IBD

Elevation in PLT count ($> 450000 \times 10^9/\text{L}$), defined as reactive thrombocytosis (RT), may frequently occur in certain conditions like hypo- or asplenicism, blood loss, acute or chronic inflammatory disorders, malignancies, and iron deficiency. The first study reporting IBD RT in 1968 by Morowitz *et al.*^[15] noted markedly-elevated concentration of circulating PLT during a period of increased clinical activity in a case series of IBD patients. This effect is the result of aberrant bone marrow throm-

bopoiesis under the influence of inflammatory mediators and the aftermath of reduced PLT lifespan due to accelerated activation and consumption of thrombocytes at the sites of inflammation.

Thrombopoiesis is mainly regulated by plasma thrombopoietin (TPO). Plasma TPO binds to C-Mpl receptors on the PLT surface, and the remaining fraction promotes thrombopoiesis by binding to the same receptors on progenitor megakaryocytes in bone marrow. Thus, in normal conditions thrombopoiesis is controlled by a negative feedback mechanism based on PLT mass in blood^[16,17]. Cytokines and other inflammatory agents, especially interleukin 6 (IL-6), promote hepatic TPO production^[18], which is considered an acute phase reactant^[19]. Heits *et al.*^[20] have shown that IBD patients with thrombocytosis have elevated plasma TPO and IL-6 levels. However, the existing data are vague, as other studies display a lack of correlation between PLT number and TPO concentration, indicating other possible regulating factors in IBD RT^[21]. Although PLT count is correlated to IBD disease activity^[22], it is not considered an independent risk factor for the increased risk of thromboembolic (TE) events observed in IBD patients as it is for cancer^[23]. Properly designed and adequately powered clinical studies evaluating predictive laboratory indices for TE events in IBD are still lacking.

Moreover, some conflicting data have emerged over the last decade about the role of preoperative RT in the occurrence of chronic pouchitis in patients undergoing ileal pouch-anal anastomosis. Two studies from the Surgery Department Division of Colon and Rectal Surgery in California have pointed out that the presence of elevated PLT count before surgery was associated with an increased risk for chronic pouchitis postoperatively^[24,25], a severe complication that can result in the removal or diversion of the pouch. In discontinuity with these studies, Lian *et al.*^[26] failed to predict the occurrence of inflammatory pouch disorders based on pre-colectomy laboratory tests, including PLT count. Larger prospectively well-designed series with patients requiring ileal pouch-anal anastomosis are needed in order to verify possible implication of PLT in this subject.

Chronic inflammatory disorders are connected to several morphological changes in PLT indices calculated in whole blood count, such as MPV, PDW, and PCT. The most widely-studied PLT parameter in humans is MPV. PLT volume decreases when an inflammatory process is present, which is mainly attributed to thrombopoiesis abnormalities and increased PLT consumption. Inflammatory mediators stimulate bone marrow precursors to enhance PLT generation at the cost of maturation time, delivering smaller PLT in circulation, while at the same time larger and more active PLT are consumed at inflammatory sites, as is proposed in the intestinal microvasculature of IBD patients^[27].

MPV changes are correlated to inflammatory disorders like myocardial infarction, stroke, diabetes mellitus, acute appendicitis, rheumatoid arthritis, chronic hepa-

titis B, celiac disease^[28-31], paroxysmal atrial fibrillation, obesity^[32], amyloidosis^[33], and retinal vein occlusion^[34]. Moreover, MPV could serve as a reliable predictor of high risk patients for portal venous thromboembolism^[35], acute coronary syndromes^[36], and stroke in patients with atrial fibrillation. MPV has also been proposed as a useful biomarker for early gastric, pancreatic, and hepatocellular carcinoma diagnosis^[37], dietary compliance to celiac disease and exacerbation of chronic obstructive pulmonary disease^[38].

In IBD patient studies a MPV value decrease has long been observed^[39] which has been inversely correlated with endoscopic and disease activity indices, such as C-reactive protein and erythrocyte sedimentation rate^[40-44]. This MPV reduction can be attributed to the decreased circulating reticulated PLT number that was found in patients with active UC compared to inactive and healthy control subjects^[44]. In line, studies have reported an inverse relationship between extent of intestinal inflammation and MPV in IBD patients^[40,41]. Öztürk *et al.*^[45] suggested that all PLT parameters (PDW, PCT, MPV) can prove to be useful surrogate markers for IBD follow-up, as they reveal strong relationship with activity indices. We observed that MPV, PCT, and PDW were correlated with certain iron deficiency markers (soluble transferrin receptors, hemoglobin) but not with activity indices such as C-reactive protein, Crohn's disease activity index score, or simple clinical colitis activity index score in IBD patients. This observation reflects a possible role of iron capacity as a regulator of megakaryopoiesis and PLT morphology^[46]. Literature reports about MPV correlations with clinical and laboratory parameters in IBD patients are presented in Table 2.

ASSOCIATION OF PLT WITH IRON DEFICIENCY IN IBD

Anemia is the most frequent extra-intestinal manifestation of IBD, affecting approximately one third of patients^[47,48]. The most prevailing type of IBD-associated anemia is iron deficiency anemia (IDA)^[48,49]. Iron deficiency is related both with up-and downregulation in PLT count, with RT reported more frequently^[50].

Several mechanisms related to iron deficiency have been implicated in PLT overproduction. Iron scarcity could trigger an increase influx of progenitor cells to the megakaryocyte cell compartment, a diminution of PLT maturation time^[51], and the generation of high ploidy megakaryocytes. Megakaryocytes can proliferate through a procedure called endomitosis, and augment DNA ploidy and cytoplasmic volume and further abandon mitosis before cytokinesis take place^[52]. Iron deficiency may lead to the production of larger polyploid megakaryocytes capable of generating numerically more PLT, as it is observed in an iron deficient rat model^[53]. Moreover, striking amino-acid sequence homology between erythropoietin (key hormone controlling erythropoiesis) and TPO, both being members of the same hematopoietic growth

Table 2 Mean platelet volume correlations with clinical and laboratory parameters in inflammatory bowel disease patients

Ref.	Disease (n)	HC (n)	MPV correlations
Yüksel <i>et al</i> ^[41]	UC (61)	27	Reduced MPV in UC compared to HC Inverse correlation between MPV and disease activity
Järemo <i>et al</i> ^[39]	UC (18), CD (9)	12	Inverse correlation between MPV and disease extent Reduced MPV in UC patients compared to HC Inverse correlation between MPV and disease activity
Güçlü <i>et al</i> ^[42]	UC (41)	(-)	Reduced MPV in active compared to non-active disease
Voudoukis <i>et al</i> ^[46]	UC (91), CD (107)	102	Reduced MPV in IBD patients compared to HC Correlation of MPV with Hb and sTfR
Öztürk <i>et al</i> ^[45]	UC (103), CD (72)	40	Reduced MPV in IBD compared to HC MPV decreases after remission in UC MPV increases after remission in CD
Kapsoritakis <i>et al</i> ^[40]	UC (93), CD (66)	38	Correlation between MPV and disease activity indices Correlation between MPV and disease extent
Kayahan <i>et al</i> ^[44]	UC (37)	20	Correlation between MPV and disease activity indices Reduced MPV in UC compared to HC
Liu <i>et al</i> ^[43]	CD (61)	50	Reduced MPV in CD patients compared to HC MPV value did not correlate to disease activity

CD: Crohn's disease; Hb: Hemoglobin; HC: Healthy controls; IBD: Inflammatory bowel diseases; MPV: Mean platelet volume; sTfR: Soluble transferrin receptor; UC: Ulcerative colitis.

factor subfamily, could be a tempting explanation for the thrombocytosis observed in children with IDA^[54] (Figure 1). This assumption, however, is in discordance with the study by Kulnigg-Dabsch *et al*^[55] that didn't observe any alteration in PLT production with the concomitant use of erythropoietin combined to iron replacement therapy in IBD patients with RT.

A special interest in IDA associated RT has arisen over the last few years in IBD^[55,56]. In a Kulnigg-Dabsch *et al*^[55] study, iron replacement was associated with dose-dependent normalization of PLT count which remained within normal range after therapy, highlighting a regulatory rather than a toxic effect of iron on PLT. Patients presented with mildly elevated or within-normal range inflammatory indices at baseline and during treatment, demonstrating that RT could be mainly attributed to IDA rather than systemic inflammatory response^[55]. In another study, iron replacement was not only associated with PLT count decrease, but also to a significant decrease in PLT activation markers, such as P-selectin and PLT-aggregation, suggesting that iron management may express anti-thromboembolic properties in IBD patients with increased risk for TE events. However, the small number of participants and the need for study protocol modification during the active phase does not allow us to make safe conclusions^[56].

Studies have also identified a correlation between PLT count, red blood cell parameters, and anemic indices in otherwise healthy IDA patients^[57,58]. In a recent study we observed a mutual relationship between PLT count and iron deficiency parameters. Inflammatory indices (C-reactive protein, Crohn's disease activity index score, and simple clinical colitis activity index score) and iron deficiency markers (ferritin, soluble transferrin receptors, and index) were correlated to PLT count in 198 consecutive IBD patients, indicating that RT is probably a multifactorial event in which iron deficiency and inflammation

hold a major role. Moreover, taking into account the low inflammatory indices in our patients' cohort, we assumed that iron deficiency could be the main factor affecting PLT count in IBD^[46].

PLT AS ACTIVE INFLAMMATORY COMPONENTS

PLT circulate at a highly-activated state in IBD, as it is demonstrated by an increased concentration of circulating PLT activation markers in the systemic circulation of patients^[59]. This activation possibly takes place in the mesenteric microcirculation, where PLT are exposed to several inflammatory mediators^[60]. Molecules in the site of injury like subendothelial collagen, cytokines from activated leukocytes, and endothelial cells, increased local adenosine diphosphate (ADP) concentration due to reduced capillary blood flow, substances released from neighboring cells, arachidonic acid, PLT activating factor (PAF), and thrombin generation augment PLT accumulation and activation in the intestinal microvasculature in IBD^[2]. During activation, PLT lose their normal discoid shape, obtain projecting forms called pseudopodia, release an increased amount of microparticles (PDMPs), and grow in size and density. Numerous metabolic reactions happen within their cytoplasm, where various inflammatory mediators are being produced^[1,39]. Proteomic studies have identified more than 300 proteins accumulated in granules of activated PLT^[61]. PLT granules are rich in PLT factor-4, β -thromboglobulin, fibrinogen, von Willebrand factor, fibrinolytic inhibitors, coagulation V and XI factors, protein S, angiogenic and mitogenic factors (PLT-derived growth factor, transforming growth factor, endothelial growth factor, and vascular endothelial growth factor), immunoglobulins, membrane ligand proteins (P-selectin), ADP, serotonin, IL-1 β , chemokines, RANTES, IL-8, and various other substances^[12]. Certain

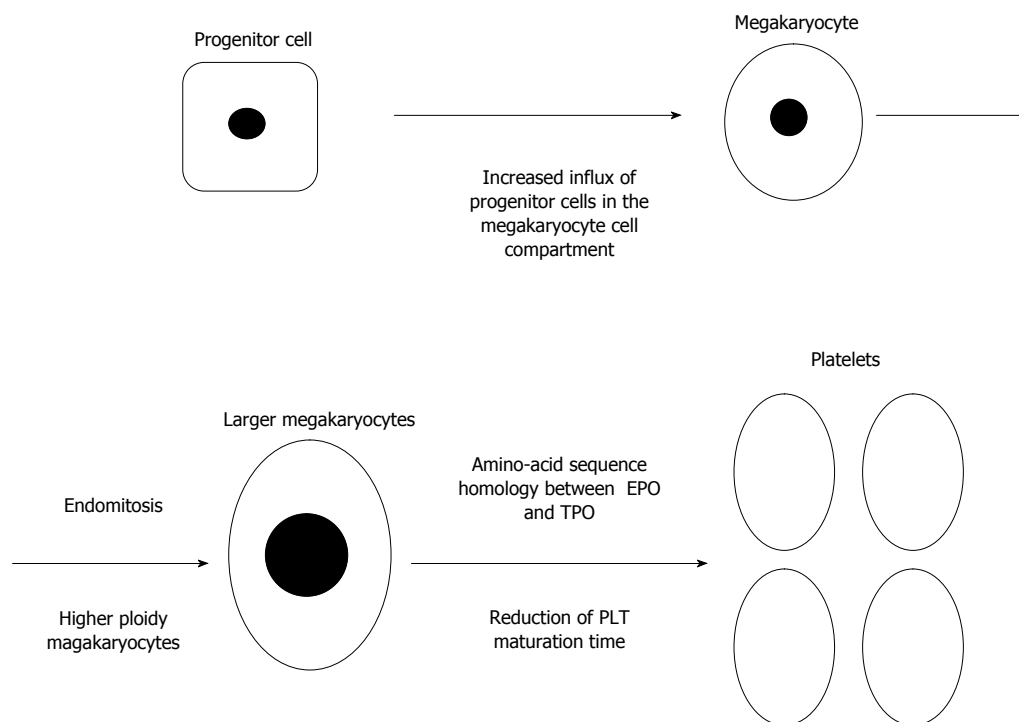


Figure 1 Possible iron deficiency mechanisms affecting platelet count in inflammatory bowel disease. PLT: Platelets; TPO: Thrombopoietin; EPO: Erythropoietin.

PLT granular products, such as P-selectin, GP IIb/IIIa, CD40L, and GP53, are incorporated into the cytoplasmic membrane, giving them a more adhesive and interacting phenotype. Moreover, PLT during activation develop receptors for chemokines, cytokines, and complement components, enabling them to participate in various inflammatory cascades in IBD^[1] (Figure 2). Molecules released from the activated PLT induce an inflammatory phenotype in endothelial cells and leukocytes. Polymorphonuclear cells enhance their superoxide, PAF, and leukotriene production, and endothelial cells stimulated by certain PLT factors (PAF, histamine, and RANTES) increase vascular permeability^[2]. CD40L(+) PLT of IBD patients induce I- and V-cell adhesion molecules (CAM) and IL-8 overexpression when co-cultured with human intestinal microvascular endothelial cells in an experimental colitis model^[62].

P selectin is a member of the CAMs family mainly produced in PLT. A soluble fraction of P-selectin is also detected in patients with inflammatory disorders, including IBD, and possibly serves as selectin binding inhibitor^[63]. The lectin containing N-terminal domain of P-selectin binds to P-selectin glycoprotein ligand (PSGL-1) found in leukocytes (mainly polymorphonuclears) mediating recruitment and rolling of infiltrating leukocytes in the gut mucosa, and initiating activation processes like chemokines production by monocytes and CD4(+) T-cells, as well as superoxide overexcretion by neutrophils^[1]. P-selectin ligation to PSGL-1 also serves in PLT-PLT aggregation and PLA formation^[2], induces tissue factor (TF) generation, and stimulates the release

of PDMPs bearing TF by leukocytes^[64]. The above mentioned findings highlight the significant role of P-selectin in IBD pathogenesis.

CD40L (CD 154) is a protein, strongly related to tumor necrosis factor (TNF) and expressed on the surface of activated PLT and immune system cells. CD40L has the ability to bind CD40 located on the surface of most immune, endothelial, and other mesenchymal cells^[65]. There are three CD40 family members encountered in humans: CD40, CD40L, and the soluble form of CD40L (sCD40L) derived by enzymatic fragmentation of CD40L in serum^[66]. The latter is believed to be produced and released only by activated PLT in IBD patients^[67]. Increased levels of CD40L(+) PLT and sCD40L are demonstrated in disorders combining inflammation and thrombosis, such as unstable angina, myocardial infarction^[68], and IBD^[67,69].

CD40L interactions have a significant role in immune mediated activation of inflammation and thrombosis. They induce TF expression by endothelial cells and monocytes^[65]. SCD40L is able to bind onto GP IIb/IIIa to promote arterial thrombosis stabilization, as was demonstrated in CD40L deficient mice^[70]. Pro-inflammatory responses of CD40L/CD40 result in chemokines, ILs, and CAMs (V-CAMs, I-CAMs, P/E-Selectin) upregulation in PLT and other immune cells^[65]. CD40L can stimulate PAF production, thus inducing PLT activation, propagating immune mediated angiogenesis in IBD in both human and murine models^[71], and provoking cytokine overexcretion by human intestinal microvascular endothelial cells such as IL-8, which constitutes a ma-

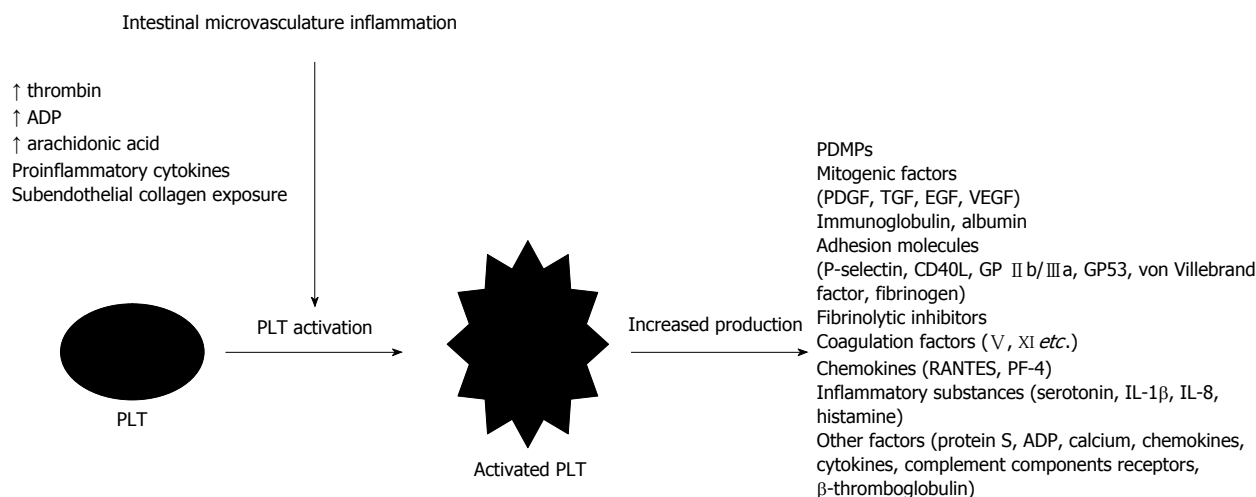


Figure 2 Factors affecting platelet function and platelet products in inflammatory bowel disease. PLT: Platelets; ADP: Adenosine diphosphate; PDMP: Platelet-derived microparticles; PDGF: Platelet-derived growth factor; EGF: Epidermal growth factor; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor; PF-4: Platelet factor-4; IL: Interleukin.

for neutrophil chemoattractant^[67]. Finally, PLT CD40L (+)-derived vesicles seem to display an immunoregulatory role by activating peripheral blood B-cells in producing immunoglobulins when co-cultured with them *in vitro*^[72] and stimulating antigen specific IgG production by germinal center modulation in the B-cell compartment^[14].

CD40L is essential in activating components of the immune system in IBD. The infiltration of neutrophils in the colonic mucosa of UC patients and macrophage chemoattraction in granulomatous lesions in CD has been found to be mediated mainly by CD40/CD40L interactions^[1]. CD40(+) immune fluorescence staining was observed only at inflamed intestinal sites and not at intact mucosal segments in intestinal endoscopic biopsies from IBD patients^[73]. A positive correlation between sCD40L and the extent of anatomical involvement in IBD was also found^[67]. Finally, CD40 deficient mice experienced significantly milder dextran sodium sulfate (DSS) colitis than wild type littermates^[74].

POTENTIAL ROLE OF PLT IN THROMBOSIS IN IBD

PLT spontaneous aggregation is a unique feature found in the blood of IBD patients that is not encountered in other inflammatory conditions^[59]. Aggregation is believed to be primarily accomplished in the mesenteric microcirculation where PLT come into close contact with increased inflammatory mediators^[60]. PLT aggregates are independent of disease activity, as their existence has been noted in colonic biopsies of IBD patients in remission, but not in healthy controls^[75].

PLT aggregation in IBD seems to represent the initial response of PLT leading to an increased risk for TE. The reported prevalence of TE events (arterial or venous thrombosis) in IBD is between 1.3% and 6.0%, with a 1.5-3.6 fold increased risk compared to the general population and other inflammatory disorders^[76,77]. The devel-

opment of TE in IBD seems to be multifactorial, with interaction of genetic and acquired factors (*e.g.*, inflammation, hospitalization, and operations). TE events in IBD indicate a higher predilection towards younger age compared to non-IBD subjects^[2]. Thromboembolism is considered a negative prognostic outcome and represents one of the four leading causes of death in these patients. Thrombosis may correlate with disease activity, but it is interesting to note that one third of the events happen during clinical remission, indicating a continuous activate state of PLT and coagulation systems in IBD^[78,79].

Moreover, the increased concentration of PLA in circulation^[80] is also considered as an aftermath of leukocyte sequestration in mesenteric circulation, where they bind to activated PLT^[81]. This interaction is mainly guided by PLT(+) P-selectin ligation to leukocytes PSGL-1. After this initial step, further ligation of PLT GP II b/IIIa to MAC-1 leukocyte membrane receptors, with fibrinogen serving as the bridging connector, intensifies binding and promotes PLA formation. PLA are major inflammatory agent carriers, more active than circulating leukocytes or activated PLT alone^[82,83] and exhibiting an enhanced ability to adhere to mucosal endothelium^[82]. Increased PLA formation is noted in many chronic inflammatory disorders like diabetes mellitus, cardiovascular and collagenous tissue diseases, asthma, systemic lupus, and rheumatoid arthritis^[84]. Therefore, PLA is indexed as a sensitive marker of inflammation and PLT activation though not in consistency with IBD activity in recent studies^[82].

PLATELET DERIVED MICROPARTICLES AND IBD

Eukaryotic cells are capable of budding small vesicles like exosomes (endosomal products), apoptotic bodies (byproducts of cell death), and MPs. Circulating MPs are a heterogeneous mixture of cellular membrane fragments that are derived from a great variety of cells, and

recapitulate the functions of their cellular origin. They influence a diverse series of physiological and pathological functions, as they can transfer genetic material (m-RNA, micro-RNA, DNA), membrane receptors, and a series of parental molecules to target cells^[85]. MP formation is a well regulated process consisting of local concentration changes in specific intracellular molecules, cytoskeleton disruption, and phosphatidylserine inversion in the outer membrane layer of ancestral cells^[86].

Although MPs are detected in low concentrations in health, a great variety of cardiovascular diseases, inflammatory disorders, cancer, and diabetes are associated with increased MP production. They are considered major procoagulant factors, due to TF and phosphatidylserine exposure on their membrane^[87]. PDMPs represent the most abundant MP population in humans, approximately 70%-90% of cell-derived MPs^[88]. Among them a large amount of PDMPs originate from megakaryocytes^[89]. PDMP production is enhanced *in vitro* by PLT agonists like Ca^{2+} , thrombin, ADP, collagen, fibrinogen, and high shear stress, confirming the statement that PDMPs are mainly derived by activated PLT^[86].

PDMPs are increased in autoimmune disorders such as mixed connective tissue disease, systemic sclerosis, primary Sjögren's syndrome, systematic lupus erythematosus, rheumatoid arthritis, Raynaud's phenomenon, and psoriasis^[87,90-92], as well as in cardiovascular diseases such as atherosclerosis, acute coronary syndrome, pulmonary embolism, and pulmonary arterial hypertension^[93-96]. Moreover, they can be used as antithrombotic indicators and side-effect markers following blood transfusion^[97,98].

Few studies have been conducted in IBD patients. Andoh *et al.*^[99] showed increased PDMPs in active IBD patients compared to inactive ones and healthy controls. PDMPs correlated with clinical disease activity indices and PLT activity markers, and significantly reduced after remission achievement. However, this study included a small sample size for exporting safe conclusions and PDMPs were measured using ELISA and not flow cytometry, the latter being considered a more reliable method. Chamouard *et al.*^[100] demonstrated that infliximab therapy induced a significant decrease in circulating MPs, mainly of PLT origin, in CD but not in UC, implicating that PDMPs shedding is important in the IBD inflammatory response. Finally, Palkovits *et al.*^[101] noted that TF(+) MP and especially TF(+) PDMPs were significantly increased in IBD patients compared to healthy controls, although they didn't correlate with markers of coagulation activity and inflammation. These results indicate that PDMPs may have an important role in IBD. Taking into account the high procoagulant and proinflammatory predisposition of PDMPs, they can be useful targets, or even vectors, of future IBD therapies.

USE OF ANTI-PLATELET DRUGS IN IBD

Anti-PLT therapy is unanimously certified as evidence-based primary and secondary prevention therapy in high

risk cardiovascular patients resulting in reduced mortality rates^[102]. Based on existing evidence, one can assume that PLT could be an ambitious target cell for IBD therapies, as it represents the critical crossroad between inflammation and coagulation.

Clopidogrel is a potent suppressor of PLT activation, PLA formation and production of PLT activation markers such as P-selectin^[103-105]. Clopidogrel significantly inhibited PLT inflammatory markers and resolved IBD symptoms in rats after a single intra-colonic administration of trinitrobenzenesulfonic acid and oxazolone^[106]. Moreover, salicylic compounds like 5-aminosalicylic acid regimens, which are broadly used in IBD, significantly reduced PLT activation markers in IBD patients^[107]. However, the use of aspirin even in a low dose in IBD is still uncertain, as it is associated with exacerbation symptoms and should be offered in patients with a strong indication for it^[108]. Larger randomized controlled studies evaluating its systematic anti-inflammatory effect in IBD are needed in order to verify possible benefits.

Azathioprine and 6-mercaptopurine are reported to inhibit collagen, ADP, and arachidonic acid-dependent PLT aggregation, as well as PLA aggregate formation^[109]. GP II b/III a antagonists (eptifibatide, abciximab, and tirofiban) have been shown to be more competent in sCD40L down regulation compared to aspirin in high risk cardiovascular patients, an observation that might be proved useful in IBD^[110]. Moreover, infliximab therapy induced significant disruption of CD40/CD40L dependent cognate interactions^[111] and reduced circulated MPs^[100] in CD patients, suggesting a potent drug effect on TNF, CD40L, and MPs production in IBD.

Other studies evaluating anti-PLT activation marker products in experimental colitis have also been conducted. CD40/CD40L pathway inhibitor (Trapidil) administration resulted in a significant reduction of colonic inflammation in wild type murine DSS induced colitis^[74]. Moreover, CD40L deficient mice exhibited a reduced thrombotic response that was restored after sCD40L administration, highlighting the possible anticoagulant effect of anti-CD40L drugs in IBD where the risk for TE events is increased^[112]. Finally, P-selectin deficient mice or P-selectin, PSGL-1 blocking antibody utilization induced significantly decreased PLT recruitment in a DSS colitis mouse model^[113].

CONCLUSION

In conclusion, there are increasing data suggesting that PLT are important key regulators in inflammatory disorders beyond hemostasis and thrombosis. Inflammation, wound repair, angiogenesis, atherosclerosis, and tumor metastasis are only some examples that reveal PLT multifactorial role. In IBD pathogenesis, PLT activation could be the missing link between inflammation and coagulation, two "independent" processes linked in such a way that each one activates and propagates the other.

Thrombocytosis has been associated with IBD mani-

festations such as disease activity, iron deficiency anemia, and development of pouchitis, whereas PLT parameters (PDW, PCT, and MPV) have been suggested as surrogate markers for IBD. PLT count increase cannot be attributed only to inflammation, as we believe that iron deficiency should be considered a major governor of thrombopoiesis. Until now, no study was designed in such a way as to discriminate to what extent inflammation and iron deficiencies are responsible for PLT increase. However, particular interest should be given to iron replacement in IBD patients, and especially those with thrombocytosis and low inflammatory indices or/and low hematocrit. The possible association between iron replacement therapy and reduction of PLT activation markers raises new questions regarding the involvement of iron scarcity in the increased incidence of TE events in IBD patients, although the data are as yet inconclusive. Additionally, PLT parameters seem to display good predictive value regarding disease activity, and can be cautiously used as cost-effective follow-up biomarkers in IBD.

Despite the increasing number of studies revealing the dominant role of PLT in IBD, little has been clarified regarding the efficacy of anti-PLT drugs in IBD. Perhaps different existing pathways between PLT hemostasis and coagulation could explain the lack of potent anti-PLT drugs approved in IBD. Breaking this vicious cycle by encountering PLT inflammation properties appears to be a challenging ordeal for future investigators and clinical physicians, who will need to come up against resisting IBD flares with a reduced selection of effective drugs.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Update on nutritional status, body composition and growth in paediatric inflammatory bowel disease

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Abstract

Growth and nutritional status are important issues in paediatric inflammatory bowel disease (IBD). While linear growth is easy to assess, nutritional status is more complicated, with reports often compromised by the use of simple measures, such as weight and the body mass index, to assess nutritional status rather than more appropriate and sophisticated techniques to measure body composition. This review is an update on what is currently known about nutritional status as determined by body composition in paediatric IBD. Further, this review will focus on the impact of biologics on growth in paediatric IBD. Significant lean mass deficits have been reported in children with IBD compared with controls, and there is evidence these deficits persist over time. Furthermore, data imply that gender differences exist in body composition, both at diagnosis and in response

to treatment. With respect to growth improvements following treatment with biologics, there are conflicting data. While some studies report enhancement of growth, others do not. The relationship between disease severity, impaired growth and the requirement for biologics needs to be considered when interpreting these data. However, key features associated with improvements in growth appear to be successful clinical response to treatment, patients in early stages of puberty, and the presence of growth failure at the onset of treatment.

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Key words: Crohn's disease; Ulcerative colitis; Lean mass; Pubertal status; Infliximab; Inflammatory bowel disease

Core tip: Assessing body composition gives a much better indication of nutritional status than measures of anthropometry, such as BMI. In children with IBD, significant and persistent deficits in lean mass, suggestive of compromised nutritional status, have been reported, both at diagnosis and following treatment. Data pertaining to body composition in response to biologics is lacking, and data concerning growth improvements is controversial. However, evidence suggests that the key components associated with linear growth improvements when treating with biologics are (1) successful clinical response to treatment; (2) patients in early stages of puberty; and (3) the presence of growth failure at the onset of treatment.

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INTRODUCTION

Treatment considerations for paediatric patients with inflammatory bowel disease (IBD) are two-fold. Firstly, to achieve optimal disease control and attain remission, and secondly, to promote growth and progression through puberty. Often, when the first consideration is achieved, the latter will follow.

Growth failure and delayed puberty have long been associated with paediatric IBD, and is more prevalent in Crohn's disease (CD) than ulcerative colitis (UC)^[1]. Malnutrition has long been described as a factor contributing to growth impairment. The development of malnutrition in this cohort is multifactorial, being influenced by enteric nutrient losses, suboptimum intake, malabsorption of nutrients, and increased energy needs^[2]. However, while linear growth is straight forward to measure, and it is clear patients with CD are more prone to growth failure than UC, assessing malnutrition is more complex, with many results limited by crude measures of weight and body mass index (BMI) as proxy measures of malnutrition. Both these measures give little information about what is actually happening in the compartments of the body, and as such, increases in these parameters may not be representative of improved nutritional status.

Assessing body composition, that is, fat mass and lean mass at its most basic, gives a much better indication of nutritional status than anthropometry. With this in mind, growth and nutritional status in paediatric patients with IBD should be considered in terms of body composition, rather than simple anthropometric changes. This review is an update on what is currently known about nutritional status as determined by body composition in paediatric IBD. Further, as there have been several recent reviews about the prevalence and mechanisms of growth failure in IBD^[3,4] this review will focus on the impact of biologics on growth in paediatric IBD. Suitable research studies were identified from the literature by searching PubMed. Key words used to search included: IBD; body composition; nutritional status; growth; child; adolescent; infliximab. Relevant studies were also identified from the reference lists of search results.

NUTRITIONAL STATUS AS DETERMINED BY MEASURES OF BODY COMPOSITION

The techniques reported in the literature to measure body composition in IBD have paralleled the technical advancements in the field and become increasing more sophisticated and, therefore, accurate. Early work utilized anthropometry, such as measurements of skinfold thickness and body circumferences, which are proxy methods at best, and are less accurate than other methods like bio-electrical impedance (BIA), isotope dilution, total body potassium and dual energy X-ray absorptiometry (DXA).

IBD compared with normative data or controls

There is general consensus in the literature that lean mass

is reduced in children with IBD compared with controls. Boot *et al*^[5] reported reduced lean mass Z-scores in a cohort of 55 children with IBD, and this reduction persisted over two years of measurement. Similarly, Sylvester *et al*^[6] also found persistence of significantly lower lean mass Z-scores over two years in patients with CD, and these remained lower than controls even after adjustment for height. Werkstetter *et al*^[7] found significantly reduced lean mass in children with well controlled IBD compared with controls, as indicated by reduced phase angle α Z-scores measured by BIA. Our group^[8] has detailed significant reductions in body cell mass Z-scores (the metabolically active component of lean mass; adjusted for height) in patients with UC having repeated measures of total body potassium over three years. Lean mass adjusted for age and lean mass adjusted for height was shown by Burnham *et al*^[9] to be significantly lower in children with CD than controls, and in a regression model including height, age, Tanner stage and race, CD was associated with a 6% reduction in lean mass. Further, concurrent increases in fat and lean mass were reported in control subjects, whereas no relationship was found in those with CD; that is, increases in fat mass were not associated with increases in lean in children with CD. Azcue *et al*^[10] report body composition comparisons between patients with CD, healthy controls, and patients with Anorexia Nervosa, however, the different techniques used to calculate fat and lean mass, and the potential errors associated with this, limit the interpretation of these data. They do suggest that their finding of an elevated ECW:ICW ratio in CD compared with control subjects is indicative of protein-energy malnutrition, which in turn, is representative of lean mass deficits.

Despite consensus with respect to lean mass reductions in IBD, not all studies are in agreement with respect to fat mass. Boot *et al*^[5] suggest proportional reductions in lean and fat mass, as shown by percentage body fat that did not differ significantly from zero in their combined IBD cohort. In contrast, in an all CD cohort Burnham *et al*^[9] report fat mass adjusted for age and fat mass adjusted for height was not significantly different from controls. Similarly, in 42 children with CD weight gain over a two-year period was explained by gains in fat mass^[6].

Several studies highlight the ineffectiveness of BMI to determine nutritional status, as compared with body composition, in this cohort. Our group^[8] have recently shown normal BMI Z-scores in patients with UC, where body cell mass Z-scores were significantly reduced. Sylvester *et al*^[6] found children with CD had lean Z-scores consistently below the mean of healthy controls over two years, despite increases in BMI after 1 year that made them comparable to healthy children. In the study of Thayu *et al*^[11], changes in body composition were not reflected by changes in BMI, shown by normalisation of BMI in the face of continued significant deficits in lean mass at follow-up for female patients with CD compared with controls. While easy to calculate, BMI is of little value in determining nutritional status in children with IBD.

Gender differences in body composition

Several studies have detailed gender differences in body composition in patients with IBD, and further, have shown different treatment effects with respect to influence on nutritional status between genders. Dung *et al*^[12] report significantly higher percentage body fat in girls with CD compared with boys, and Sentongo *et al*^[13] detail girls with CD have significantly higher percentage body fat (approximately 6%) than controls, while boys were not different. However, for any given age, this study found significantly reduced lean mass in both the boys and girls with CD, which was associated with disease activity.

Work by Thayu *et al*^[11,14] is somewhat in disagreement with the body composition patterns described above. In contrast to the studies of Dung *et al*^[12] and Sentongo *et al*^[13], Thayu's group studied incident CD within two weeks of diagnosis, and report body composition at diagnosis^[14] and changes over time in response to treatment^[11] in the same cohort. At diagnosis, girls with CD displayed a decrease in both lean and fat mass compared with controls (wasting), while boys displayed reductions in lean mass, with relative preservation of fat mass (cachexia). After adjustment for race, Tanner stage, age and fat mass for height Z-scores, deficits in lean mass remained significant in both genders compared with controls, but were more pronounced in the girls with CD compared with boys, and within the girls, in the girls diagnosed during adolescence. Interestingly, body composition was not associated with disease activity, however, there were correlations with inflammatory markers. A subset of this cohort was followed for 24-63 mo, and in boys the body composition pattern changed from cachexia to one of normalised lean mass, but excess fat mass compared with controls. In girls, wasting at baseline developed into cachexia at long term follow-up, illustrated by continued deficits in lean mass, but normalisation of fat mass compared with controls.

An important consideration when interpreting these data detailing gender differences in body composition is the differential timing of peak height velocity between girls and boys^[15] and the changes in body composition that are associated. Gender differences may in part be explained by the timing of onset of disease in relation to the occurrence of peak height velocity^[13], which, in normal developing children, occurs earlier in girls than boys.

Effects of treatment on body composition

Data describing treatment effects of medications for IBD is somewhat confounded by disease severity. For example, disease activity was correlated with greater lean mass deficits in the study of Burnham *et al*^[9], and there was a trend for use of corticosteroids to be associated with lean mass reduction, which may simply be a result of increased corticosteroid use with more severe disease. The same study found mesalamine was predictive of lean mass for height Z-score less than -1.00, and the authors suggest this is indicative of upper gastrointestinal disease, which is associated with more micronutrient deficiencies,

and hence, may compromise nutritional status.

Nonetheless, Thayu *et al*^[11] described determinants of change in body composition during follow-up of their CD cohort. Medications included corticosteroids, methotrexate, 6-mercaptopurine, azathioprine, infliximab and enteral nutrition (not exclusive). In their model of predictors, greater improvements in lean mass for height Z-scores were associated with concurrent infliximab, while greater increases in fat for height Z-scores were associated with cumulative corticosteroid dose and methotrexate. Interestingly, high dose corticosteroid therapy has been shown to significantly increase whole body protein breakdown and loss, even in the short term, in children with CD and this may influence lean body mass acquisition in the long term^[16]. This potentially explains the persistent deficits in lean mass over time described by Thayu *et al*^[11] and Sylvester *et al*^[6].

Body composition in response to nutritional therapy

The early study of Lin *et al*^[17] combined measures of subscapular and triceps skinfold thickness, mid arm circumference, CT scanning of the thigh, and creatine excretion to investigate truncal and extremity body composition in children with both CD and UC combined. Normative data were not reported, nor were measures converted to Z-scores, but rather, their work investigated change in response to two durations of parenteral nutrition. Both short term (ST; 5 wk) and long term (LT; 10 wk) parenteral nutrition were associated with reduced disease activity and significant increases in weight, muscle mass, and truncal fat. Further, height was significantly increased at 50-d post cessation of ST nutrition and at cessation of LT, however, the relevance of this is limited as only absolute heights were given and not Z-scores (no control group). It is, therefore, unknown whether the increase in height was simply a reflection of normal growth, as opposed to increased growth, as there was no comparison group. With respect to extremity composition, increases in fat were more pronounced in the arms and increases in muscle were more pronounced in the legs, with changes more apparent with longer parenteral nutrition.

Investigating two types of exclusive enteral nutrition (EEN), Khoshoo *et al*^[18] also showed improved body composition and decreased disease activity. Fourteen children with CD increased weight, lean mass (BIA) and triceps skinfold thickness after both three and six weeks of EEN compared to baseline. Similarly, Azcue *et al*^[10] showed improvements in weight, percentage ideal body weight and absolute values of lean mass in children with CD on EEN. EEN was compared to corticosteroids and both groups significantly increased in the aforementioned parameters. In an age and Tanner stage matched subgroup of ten males, height was shown to significantly increase in the EEN group compared with the corticosteroid group. Interestingly, in both groups percentage of lean mass did not change significantly over the three months of treatment, but percentage fat mass did, with a trend to greater increase in the corticosteroid group.

This finding is perplexing, as for percentage fat mass to increase there would need to be a decrease in percentage lean mass. However, as previously mentioned several different body composition techniques, of varying accuracy and sophistication, were used to measure each component, and as there is error and assumptions inherent in different techniques, this questions the validity of their body composition component data comparisons. For example, Boot *et al*^[5] have shown that BIA overestimates fat mass compared with DXA as the standard in a cohort of IBD patients. They also found greater differences between the two methods when DXA determined bone mass and lean tissue mass were added together and compared with lean mass by BIA. Further, Sentongo *et al*^[13] have shown significant differences between lean and fat mass predicted from skinfold thickness compared with assessment by DXA.

GROWTH IN THE ERA OF BIOLOGICS

In the era of biologics, initial investigations into the efficacy and safety of their use in paediatrics are now evolving into interest in their ability to promote growth (Table 1) and improve nutritional status, although data predominantly investigate weight or BMI change and data on body composition are scarce. In three retrospective studies^[19-21], weight following infliximab therapy was shown to increase, but no significant changes in linear growth were reported. Afzal *et al*^[19] reviewed the case notes of 24 children and detailed growth parameters 6 mo prior to the first infusion of infliximab, at the time of first infusion, and 6 mo post third infusion. All children were on concomitant immunosuppression and while weight Z-score significantly improved from initial dose of infliximab to 6 mo post, no significant change in height Z-score was found between time points. Similarly, Sinitsky *et al*^[21] reported a significant improvement in BMI Z-score and a trend to improvement in weight Z-score at 1 year after starting infliximab in a cohort of 16 patients, however, height Z-scores were not different. Diamanti *et al*^[20] retrospectively evaluated 28 patients and divided them into groups according to therapy so as to compare combined infliximab, mesalazine and azathioprine, with mesalazine and azathioprine only. Significant increases in weight and BMI between baseline and follow-up (median 10 mo) were reported for the infliximab group, however, height was not found to be different in either treatment group.

Pfefferkorn *et al*^[22] described the relationship between growth and current treatment options in children remaining in Tanner stages 1-3 over 2 years. Thirty-six percent of their cohort received infliximab and no significant differences in height velocity Z-scores were found at one or two years follow-up. More frequent doses of infliximab were reported in children receiving early and sustained corticosteroid use, and this association persisted over the two-years of follow-up.

In contrast, other studies have reported resumption of normal linear growth following treatment with biologics^[23-29]. In a small number of patients with CD

($n = 6$) who were refractory to conventional therapy (corticosteroids and/or azathioprine) and had growth impairment (at least -1.00 change in Z-score for height), de Ridder *et al*^[25] described recommencement of normal linear growth velocity in half their retrospectively studied cohort. Borrelli *et al*^[23] prospectively studied 18 children with severe CD and reported both significantly increased weight and height Z-scores at 6 mo post induction regimen. Following the three induction infusions, endoscopic and histologic scores were significantly decreased. Clinical remission was achieved in 10 patients and inflammatory remission in 12 patients, and eight patients who had achieved both clinical and inflammatory remission had retreatment with infliximab beyond the induction regimen. When examining retreated patients compared with the 10 who only completed induction therapy, it was shown that the significant improvements in weight and height Z-scores remained only in the retreated group. It is interesting to note that mean height Z-scores were indicative of growth failure in the retreated group (-1.15), whereas they were not in the induction group (-0.86). Further, all patients in the retreated group displayed clinical and inflammatory remission post induction therapy. Hyams *et al*^[26] studied only patients who clinically responded to induction with infliximab in their randomised controlled trial of two different dosage regimens. Height Z-scores were determined only in those patients with greater than a 1 year delay in bone age, and at both wk 30 and 50 height Z-scores were significantly improved.

Pubertal progression and skeletal maturation are important considerations when evaluating the impact of therapies on growth. Both these parameters were taken into account by Walters *et al*^[29] in their retrospective investigation of growth during the first year of infliximab therapy. A bone age correction factor was applied to Z-score calculations for those children with a delay and patients were grouped according to pubertal status (Tanner stages 1-3 *vs* Tanner stages 4-5). All 27 patients with growth assessed established at least a partial response to the induction regimen, and mean height Z-score had decreased over the period from diagnosis to infliximab induction, even with the use of other conventional therapies. Height and height velocity Z-scores were subsequently found to improve only in those patients in early puberty, however, all children showed significant improvement in weight. Improvements in height velocity, weight and BMI were significantly greater in those children exhibiting complete symptomatic remission as opposed to partial. Similar results with respect to pubertal status and clinical response were reported in the retrospective study of Malik *et al*^[28]. Height velocity Z-scores accounted for pubertal status, and height and height velocity Z-scores significantly improved over the first 6-mo of treatment, with height Z-scores additionally showing significant increases 12-mo from baseline. Clinical responders showed significant improvements in height velocity. In a prospective study of children with severe refractory or corticosteroid dependent CD, ten children who had not completed pubertal growth showed significant improvement in height Z-score

Table 1 Summary of studies investigating the impact of biologics on linear growth

Ref.	Study type and biologic	Subjects and medication at baseline	Growth failure ¹	Pubertal status data	Measurement times	Remission achieved	Linear growth outcomes
Afzal <i>et al</i> ^[19]	Retrospective; infliximab	<i>n</i> = 24; median age: 10.3 yr; All concomitant immunosuppression	No	<i>n</i> = 0 in Tanner 5	T - 6; T0; T + 6 post 3 rd infusion	<i>n</i> = 17 clinical remission after 3 rd infusion; of these, <i>n</i> = 14 relapsed and required further infusions	No sig Δ ht Z at T + 6
Diamanti <i>et al</i> ^[20]	Retrospective; infliximab	<i>n</i> = 28; median age: 13 yr in infliximab, 5-ASA and azathioprine (Group A: <i>n</i> = 14); 14 yr in 5-ASA and azathioprine (Group B: <i>n</i> = 14)	Data not given	Data not given	T0; median 10 mo post	Clinical remission in group A	No sig Δ HV Z at 10 mo post for either group
Sinitsky <i>et al</i> ^[21]	Retrospective; infliximab	<i>n</i> = 16; mean age 13.0 yr; <i>n</i> = 2 concomitant MTX; <i>n</i> = 1 6-MP; <i>n</i> = 1 tacrolimus; <i>n</i> = 8 5-ASA; <i>n</i> = 14 azathioprine; <i>n</i> = 7 corticosteroid	No	Data not given	T0; T + 12	<i>n</i> = 10 clinical remission	No sig Δ ht Z at T + 12
Pfefferkorn <i>et al</i> ^[22]	Prospective; infliximab	Subgroup <i>n</i> = 34 commencing infliximab during first year of study; mean age: data not given; concomitant medication: data not given	No	Tanner 1-3	Dx; T + 12; T + 24	Data not given	No sig Δ HV Z at T + 12; No sig difference HV Z at T + 24 between infliximab ≥ 1 yr, vs < 1 yr or no infliximab
Borrelli <i>et al</i> ^[23]	Prospective; infliximab	<i>n</i> = 18; median age: 13 yr; <i>n</i> = 18 concomitant azathioprine; <i>n</i> = 15 mesalamine; <i>n</i> = 13 corticosteroids	Yes in retreated group only	No	T0; T + 6	After induction <i>n</i> = 10 clinical remission; <i>n</i> = 12 inflammatory remission. <i>n</i> = 8 were retreated	Sig ↑ ht Z from T0 to T + 6 in retreated group only; Note: all in retreated group had achieved clinical and inflammatory remission
Cezard <i>et al</i> ^[24]	Prospective; infliximab	Subgroup <i>n</i> = 10; mean age: data not given; concomitant medication: data not given	No	Pubertal growth not completed	T-12; T + 12	Data not given	Sig ↑ HV Z at T + 12
de Ridder <i>et al</i> ^[25]	Retrospective; infliximab	Subgroup <i>n</i> = 6 of refractory group; mean age: 13.8 yr; of these, <i>n</i> = 6 concomitant immunosuppression; <i>n</i> = 4 corticosteroids	Yes	No	Collection points unclear: patients followed for 8-122 mo	<i>n</i> = 3 good response; <i>n</i> = 2 became unresponsive at second infusion; <i>n</i> = 1 ceased due to allergy	<i>n</i> = 3 resumed normal linear growth velocity, all of which were in good response group; <i>n</i> = 3 no change
Hyams <i>et al</i> ^[26]	Prospective; infliximab, randomized to 8 or 12 weekly infusions	<i>n</i> = 103; mean age: 13.3 yr; however, ht Z only assessed in those with > 1 yr delay skeletal maturation (<i>n</i> = not reported); <i>n</i> = 93 concomitant 6-MP/azathioprine; <i>n</i> = 9 MTX; <i>n</i> = 56 5-ASA; <i>n</i> = 36 corticosteroids	Yes	> 1 yr delay skeletal maturation	T0; week 30; week 54	All displayed clinical remission to induction regimen prior to randomization	Sig ↑ ht Z from T0 to weeks 30 and 54
Malik <i>et al</i> ^[27]	Retrospective; adalimumab	<i>n</i> = 36; median age: 14.7 yr; of these, <i>n</i> = 34 prior infliximab (<i>n</i> = 7 non-responders; <i>n</i> = 16 loss of clinical response; <i>n</i> = 11 allergic reaction); <i>n</i> = 23 concomitant immunosuppression; <i>n</i> = 15 corticosteroids	No	<i>n</i> = 17 Tanner 1-3; <i>n</i> = 11 Tanner 4-5	T0; T + 6; <i>n</i> = 11 T + 12	<i>n</i> = 28 clinical remission	Sig ↑ ht Z and HV at T + 6 for whole group, those in clinical remission, Tanner 1-3, immunosuppression, allergic reaction to infliximab; no sig changes for group followed to T + 12; independent of corticosteroid use
Malik <i>et al</i> ^[28]	Retrospective; infliximab	<i>n</i> = 28; median age: 13.1 yr; <i>n</i> = 17 concomitant 5-ASA; <i>n</i> = 13 azathioprine; <i>n</i> = 13 MTX; <i>n</i> = 12 corticosteroids	Yes	<i>n</i> = 20 Tanner 1-3	T - 6; T0; T + 6; <i>n</i> = 25 T + 12	<i>n</i> = 21 clinical response; <i>n</i> = 10 clinical remission	Sig ↑ ht Z from T0 to T + 6, and T - 6 to T + 12 for whole group; Sig ↑ HV from T0 to T + 6 for whole group, clinical responders, Tanner 1-3, no corticosteroids, MTX throughout

Walters <i>et al</i> ^[29]	Retrospective; <i>n</i> = 27; median age: 14.3 yr; <i>n</i> = 3 infliximab concomitant corticosteroids; <i>n</i> = 25 immunosuppression	Yes	<i>n</i> = 9 delayed skeletal maturation; <i>n</i> = 19 Tan- ner 1-3; <i>n</i> = 8 Tanner 4-5	T0; T + 12; median 26 mo post (cur- rent)	<i>n</i> = 20 clinical remission; <i>n</i> = 7 partial remission	Sig ↑ HV from T0 to T + 12 for Tanner 1-3 (and this group displayed growth failure). Within Tanner 1-3, sig ↑ HV from T0 to T + 12 for complete remission; Sig ↑ ht Z from T0 to current for Tanner 1-3; ht Z negatively correlated with disease duration
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¹Growth failure defined as mean group height Z-score < -1.00 at pre or initial biologic infusion. ASA: Aminosalicic acid; MTX: Methotrexate; 6-MP: 6-mercaptopurine; Dx: Diagnosis; T - 12: 12 mo pre commencement; T - 6: 6-mo pre commencement; T0: Commencement of biologic; T + 6: 6 mo post commencement; T + 12: 12 mo post commencement; ht: Height; HV: Height velocity; sig: Significant at *P* < 0.05; Δ: Change; ↑: Increase; Z: Z-score.

in the year after treatment compared to the year before^[24]. In the whole group of 21 children, 90% achieve complete remission.

A further study by Malik *et al*^[27] detailed the effects of a different biologic on growth in children with CD, namely adalimumab. Their cohort comprised mainly of children (34 out of 36) who had previously been treated with infliximab but were either unresponsive, lost clinical response or had an allergic reaction. Both height Z-score and height velocity significantly improved over 6 mo, however, this increase was significant only in the group who achieved clinical remission. Further, height Z-score did not show significant change in those patients who were either unresponsive or lost clinical response to infliximab, but was only apparent in those with an allergic reaction to infliximab. Linear growth was also related to stage of puberty, with only those in the early stages of puberty (Tanner 1-3) showing significant increases in height Z-score and median height velocity, and while use of corticosteroids did not impact improvements in height, those on concurrent immunosuppression displayed significant improvement as opposed to those who were not.

In summary, growth deficits are a marker of more severe disease^[3], as is use of biologics^[30]. Hence, the relationship between treatment with infliximab and growth promotion seems multifactorial. From the data reviewed herein, features associated with improvements in growth with use of biologics appear to relate to clinical response to treatment, stage of puberty, and presence of growth failure. Evidence suggests that clinical response is important for improving growth and while limited data exist, this is probably related to mucosal healing^[23]. It is also apparent, and not surprising, that children late in puberty do not respond with linear growth improvement. This may have been a factor associated with the studies not showing improvement in height as pubertal status was either not assessed^[20,21], or indicated to be in the later stages^[19]. Better growth response is also seen in those patients who are suffering from growth failure prior to treatment, with studies showing no improvement involving a cohort where growth was not impaired^[19,21]. The study of Diamanti *et al*^[20] is limited by the authors only looking at change in actual height values, with both genders grouped together, and no information on pubertal status. Hence, it is difficult to determine at what stage

their patients are with respect to pubertal progression and peak height velocity.

CONCLUSION

Nutritional status, as indicated by compromised body composition (that is, reduced lean mass), is present in children with IBD and persists over time, irrespective of treatment. Further, alterations in body composition are expressed differently between boys and girls, and in response to treatment. Reports suggest girls present with wasting which morphs into cachexia with treatment. In contrast, boys present with cachexia, with resolution of lean mass with treatment, and excess of fat mass. It must be noted that literature in this area is relatively limited, and more studies are needed, particularly addressing responses to treatment.

As with compromised nutritional status, growth deficits are reported in children with IBD. Data are promising with respect to improvements in linear growth as a result of treatment with biologics, however, it is clear that further research is necessary in this area as the majority of studies conducted are retrospective in nature and subject numbers are small. Key features associated with improvements in growth appear to be successful clinical response to treatment, patients in early stages of puberty, thereby allowing a greater window of opportunity for growth potential, and the presence of growth failure at the onset of treatment, again allowing for greater growth potential. An area that is lacking for evidence is the impact of biologics on body composition, and more data are warranted in this area.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Inflammatory bowel disease course in Crohn's disease: Is the natural history changing?

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Abstract

Crohn's disease (CD) is a multifactorial potentially debilitating disease. It has a variable disease course, but the majority of patients eventually develop penetrating or stricturing complications leading to repeated surgeries and disability. Studies on the natural history of CD provide invaluable data on its course and clinical predictors, and may help to identify patient subsets based on clinical phenotype. Most data are available from referral centers, however these outcomes may be different from those in population-based cohorts. New data suggest the possibility of a change in the natural history in Crohn's disease, with an increasing percentage of patients diagnosed with inflammatory disease behavior. Hospitalization rates remain high, while surgery rates seem to have decreased in the last decade. In addition, mortality rates still exceed that of the general population. The impact of changes in treatment strategy, including increased, earlier use of immunosuppressives, biological therapy, and patient monitoring on the natural history of the disease are still conflictive. In this review article, the authors summarize the available evidence on the natural history,

current trends, and predictive factors for evaluating the disease course of CD.

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Key words: Crohn's disease; Natural history; Surgery; Mortality; Disease course; Inflammatory bowel disease

Core tip: Studies on the natural history of Crohn's disease (CD) provide invaluable data on its course and clinical predictors, and may help to identify patient subsets based on clinical phenotype. New data suggest the possibility of a change in the natural history in CD, with an increasing percentage of patients diagnosed with inflammatory disease behavior. Hospitalization rates remain high, while surgery rates seem to decrease in the last decade. Mortality rates still exceed that of the general population. The impact of changes in treatment strategy, including increased, earlier use of immunosuppressives, biological therapy, and patient monitoring on the natural history of the disease are still conflictive.

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INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract of unknown etiology. Both genetic and environmental risk factors (*e.g.*, smoking or appendectomy) contribute to its pathogenesis^[1]. During the past two decades, the incidence pattern of inflam-

matory bowel disease (IBD) has changed significantly^[2]. The disease course is reported to be highly variable, but the majority of patients eventually develop penetrating or stricturing complications. Nevertheless, there are still relatively limited data available on the natural history of IBD from population-based studies.

The phenotypic classification of CD based on clinical features plays an important role in patient management, and may help predict the clinical course in CD patients^[3]. In 2005, the Montreal revision of the Vienna classification system was introduced^[4]. The broad categories for CD classification remained the same [terminal ileum (L1), colon (L2) and ileocolon (L3) and upper gastrointestinal (GI) (L4) as modifier], behavior [non-stricturing non-penetrating (B1), structuring (B2) and penetrating (B3)] with some changes: *e.g.*, upper GI disease and perianal involvement became modifiers classified independently of, or alongside, disease at more distal locations and the later with disease behavior. Current practice guidelines from European Crohn's and Colitis Organisation advocate the use of the Montreal classification in both CD and ulcerative colitis (UC)^[5]. Using the Vienna classification system, it has been shown in referral IBD cohorts that a significant change in disease behavior often occurs over time, whereas disease location remains relatively stable^[6,7]. It is still uncertain whether this progression is preventable.

Other significant adverse outcomes include need for hospitalization, surgery, and reoperations. Hospitalization and surgery are considered to be markers of disease severity in CD and are associated with high costs^[8]. There are relatively limited data available on hospitalization trends, and data interpretation is complicated by local management strategy and reimbursement issues. According to recent population-based studies, major surgery was required in 40% to 50% of CD patients within 10 years of diagnosis in the last 2 to 3 decades, with postoperative recurrence rates as high as 50% at 10 years. However, new data suggest that surgical rates already began to decrease prior to the widespread use of biologicals. The ultimate negative outcome is mortality^[9]. CD mortality is still higher than that of the background population and current data do not suggest a change.

Recently, Peyrin-Biroulet *et al.*^[10] published a systematic review of the natural history of CD in population based-cohorts. According to the authors' conclusions, available data did not suggest a significant change in CD outcome, with approximately half of patients requiring surgery within 10 years of diagnosis. Furthermore, the authors stated that the impact of changing treatment paradigms with the increased use of immunosuppressants and biological agents on the natural history of the disease was poorly understood. In this article, evidence regarding the natural history, the current trends in outcomes and predictive factors for evaluating the disease course in CD, are discussed and summarized.

DISEASE LOCATION, BEHAVIOR AND OVERALL DISEASE ACTIVITY: CHANGING PATTERNS OR DIFFERENCES DUE TO DIAGNOSTIC TOOLS, AGE AT ONSET, GEOGRAPHIC REGION AND HOSPITAL SETTING?

In CD, disease location at diagnosis is relatively homogeneous and stable, with the exception of the reported variance in the frequency of upper gastrointestinal location, especially when comparing pediatric- and adult-onset populations. In addition, according to some studies, the proportion of isolated colonic disease seems to have increased in the last decade. In the recent study by the IBSEN group^[11], 27% of patients had L1 disease, 48% L2, 23% L3, and 2% L4 disease at presentation. Somewhat lower rates of isolated colonic disease were reported from Denmark (L2: 30%, 43%, and 37%, in 1962-1987, 1991-1993 and 2003-2004, respectively)^[12]. Similar data were recently reported from Eastern Europe (L1: 20%; L2: 35%, L3: 44%, and L4: 2.4%) in 2002-2006^[13]. Somewhat lower frequency of ileocolonic disease was reported from the Mayo Clinic^[14]. Disease extent was ileal in 45.1%, colonic in 32.0%, and ileocolonic in 18.6%.

Finally, two very recent, multinational, population-based cohorts have come to similar conclusions. In the EpiCom study^[15], the distribution was not significantly different in centers from Western and Eastern Europe (L1: 35% *vs* 43%, L2: 31% *vs* 24%, L3: 27% and 32%, isolated L4: 8% and 2%, total L4 involvement: 24% *vs* 17%). The frequency of total upper gastrointestinal involvement was higher compared to previous reports. Another study was published from eight countries across Asia and Australia^[16]. Interestingly, disease location was very similar in the Asian countries and Australia (L1: 31%, L2: 24%, L3: 45%, and L4: 5%). The highest variability is reported in the rate of upper gastrointestinal involvement. This may be at least partly associated with diagnostic procedures (*e.g.*, completeness of bowel investigation), but differences in the definitions and interpretation of minute upper gastrointestinal lesions. As an example, in the recent EpiCom study, only 10%-34% of adult onset patients underwent a gastroscopy, while a full colonoscopy was performed in 93%-96%. Additional small bowel imaging (capsule endoscopy, magnetic resonance imaging, or computed tomography) was performed approximately 60% of CD patients. Of note, location seems to be relatively stable with only 10%-15% change after approximately 10 years' follow-up^[7,17,18].

Up to one-third of patients present with complicated disease phenotype at diagnosis. In the IBSEN cohort, 36%, 49%, and 53% of patients presented with stricturing or penetrating disease at diagnosis or developed such complications within 5 or 10 years. However, some recent studies also reported a change in the initial disease behavior over time. In the Veszpremi cohort, patients

diagnosed from 1999 to 2008 presented more frequently with inflammatory disease behavior compared to the previous cohort (65% *vs* 50%)^[18]. Similarly, the probability of progression to complicated disease behavior was associated with the calendar year of diagnosis, but not with age at onset; after five and seven years 15.1% and 21.8% of patients diagnosed after 1998 progressed to complicated disease, while 27.4% and 33.3% of patients diagnosed between 1977 and 1998 showed such a progression. Other factors identified were disease location, perianal disease and smoking.

Recently, authors from New Zealand^[17] published a population-based cohort study, showing that > 70% of CD patients had inflammatory disease at diagnosis, while only 23% and 40% of patients with initial inflammatory disease progressed to complicated disease phenotypes after five and ten years of follow-up, respectively. The median follow-up for CD patients was, however, only 6.5 years. In a study from the Mayo Clinic, 81.4% had non-stricturing, non-penetrating disease, 4.6% had stricturing disease, and 14.0% had penetrating disease at diagnosis^[14]. Similarly, only 22% of patients had fistulizing complications in the Manitoba CD cohort^[19]. The cumulative risk of developing either complication in the Mayo cohort was 18.6% at 90 d, 22.0% at 1 year, 33.7% at 5 years, and 50.8% at 20 years after diagnosis. Similarly, B1 behavior was observed in 68% and 75% of patients in Western and Eastern Europe, respectively in the EpiCom study^[15] with 10% of all patients presenting with perianal involvement. The rate of inflammatory disease behavior was even higher in Australian patients in the ACCESS study^[16] (Australia: 88% *vs* Asian countries: 66%), with similar perianal involvement (12% and 18%). Another remarkable finding of this study was that UC incidence increased parallel with age. Nonetheless, some of these changes may result from bias due to diagnostic delay, differences in the diagnostic tools and completeness of bowel examination in the different time periods.

In contrast, in the landmark study by Cosnes *et al*^[6], up to 70% of CD patients developed either penetrating or stricturing disease within 10 years of diagnosis in a referral CD cohort. Similar results were published in a Belgian referral cohort^[17]. During 10 years' follow-up, 45.9% of patients had a change in disease behavior from non-stricturing, non-penetrating disease to either stricturing (27.1%) or penetrating (29.4%) phenotypes. In contrast, disease location remained relatively stable during follow-up, with only 15.9% of patients exhibiting a change in disease location within 10 years. The rate of perianal complication varies between 10%-20% at presentation. Of note, these were referral center cohorts and as highlighted earlier, trends were to some extent different in the population-based setting.

According to the available literature, pediatric-onset CD runs a more aggressive course, with more extensive disease location, more upper GI involvement, more active disease, growth failure, and need for more aggressive medical therapy in predominantly referral-center stud-

ies^[20-22]. While data on overall disease course so far have lacked consensus, pediatric disease behavior seems to parallel that of adults^[23]. A Scottish study simultaneously compared disease behavior and location in pediatric and adult onset IBD patients^[24]. In childhood-onset patients a clear difference in disease location at onset and after five years exists; with less ileum- and colon-only location but more ileocolonic and upper gastrointestinal involvement among pediatric-onset patients ($P < 0.001$ for each). In addition, disease behavior after five years did not differ between the two groups. Similar trends were recently reported from the Eurokids registry with a larger proportion of pediatric-onset patients presenting with extensive disease (L1: 16%, L2: 27%, L3: 53%, and L4: 54%)^[22]. Finally, according to French data, pediatric-onset CD was characterized by frequent occurrence of a severe phenotype during follow-up, with extensive location, complicated disease, and frequent need for immunosuppressives^[25].

Additionally, according to the findings by Pigneur *et al*^[21], patients with childhood-onset CD often have more severe disease, increased frequency of active periods, and increased need for immunosuppressants. In contrast, the cumulative risks of stricturing and penetrating complications and need for surgery were not different between childhood-onset and adult-onset patients. Similar findings were reported recently from a population-based study including both pediatric and adult onset cohorts from Hungary^[18] and another from Canada^[26]. Interestingly, in the most recent publication from the EPIMAD registry^[27], patients with pediatric-onset disease had roughly similar disease behavior at diagnosis compared with patients with an age at onset between 17-39 years, 40-59 year or > 60-years (B1: 72%, 66%, 69% and 78%). In this paper, pediatric-onset patients presented more frequently with ileocolonic disease, while elderly-onset CD patients (> 60 years at diagnosis), isolated colonic disease. In addition, complicated disease developed significantly more frequently in the pediatric-onset patients compared to patients with an elderly onset (50% *vs* 30% at maximal follow-up). The disease course in elderly-onset patients was altogether milder^[28]. Similar findings were reported also from Hungary^[29].

Few data are available regarding relapse rates and overall disease course in IBD. Most data were published from the Nordic countries. In one early publication, long-term disease course was reported in 185 CD patients followed-up regularly between 1960-1978 in Copenhagen^[30]. About 45% of patients were clinically asymptomatic for all observation years. The disease activity was low in approximately 30% of patients and moderate-to-high in approximately 25%. Continuous disease activity was observed in about 20% of patients and intermittent symptoms were reported in 35% of those with active disease in a given year. However, the cumulative relapse rate after five years reached 93.1%. Similar disease course was reported in a follow-up cohort from the same region in 1991-1993^[12].

Somewhat different rates were published in the EC-

IBD study^[31]. All-type first cumulative recurrence rates were 34%, 69.2%, and 77.5% after 1, 5, and 10 years of follow-up, respectively, in 358 CD patients, with similar second and third all-type relapse rates (40.2%, 76.9% and 82.6% *vs* 45.9 and 76.4% after 1, 5, and 10 years). Upper gastrointestinal location and therapy with 5-aminosalicylic acid therapy were associated with increased risk of relapse. Interestingly, relapse rates were associated with the geographic region. Higher relapse rates were reported from Copenhagen, while lower rates were observed in Greece, Italy, and Norway. Similar to earlier reports, high cumulative relapse rates (53%, 85% and 90% after 1, 5, and 10 years, respectively) were reported recently from the IBSEN group^[11]. This was associated with early need for steroids but not with disease phenotype or smoking habits. In contrast, approximately 44% of patients were in clinical remission during the second five-year period and 43% experienced a decrease in disease severity (according to predefined disease patterns) during the follow-up period. In contrast, 3% of patients experienced an increase in severity, 19% experienced chronic continuous symptoms, and 32% experienced a relapsing course.

HOSPITALIZATION: IS THIS AN OBJECTIVE MEASURE?

Although hospitalization is an important outcome measure, it is subject to inconsistency, as it is influenced by multiple factors other than disease severity, such as the need for diagnostic workup, health insurance reimbursement policies and ethnic differences. In addition, the threshold for hospitalization varies between specialized centers, community hospitals, and private practice. In addition, a restructuring of costs is currently seen, as highlighted in a short-term study from The Netherlands^[32]. In this study, tumor necrosis factor inhibitors (anti-TNFs) accounted for as much as two-thirds of the direct costs in CD and one-third in UC (with a three-month total cost of 1626€ in CD and 595€ in UC). Future studies are needed to investigate if tight control and aggressive therapy based on early patient profile stratification leads to superior long-term outcomes. A cost-benefit analysis is also required to justify the cost burden of these medications.

Relatively few data are available regarding hospitalization rates in patients with CD. Several decades ago, a significant proportion of diagnostics were performed on an inpatient basis, leading to fairly high initial hospitalization rates as reported in Scandinavia. For example, in Copenhagen the hospitalization rate in the year of diagnosis 83% in CD patients from 1962 to 1987. In addition, approximately 20% of patients were admitted yearly over the next five years^[33]. Data from the 1990s is available from a pan-European prospective follow-up study^[34]. This study confirmed that hospitalization rates declined significantly from the second year after diagnosis. The cumulative risk of overall hospitalization was also lower compared to the previous year (52.7% at 10 years from

diagnosis) but with considerable differences between countries. Rates were highest in Denmark, Ireland, Portugal while low rates were observed in Norway, Greece, and Italy.

Likewise, high hospitalization rates were reported in a population-based study from Canada^[35]. In 1994-2001, approximately 25% of subjects with Crohn's disease were admitted annually. The annual hospitalization rate declined from 29.2 to 26.9 per 100000 over the seven years of the study. The readmission rate was 39.4%, with almost half of the hospitalizations occurring for surgery. In a more recent population-based study from the same region^[36] the authors reported stable hospitalization rates in CD patients diagnosed between 1988 and 2008, with the highest hospitalization rates within the first year of diagnosis (approximately 1.3 admissions per person-year). Similar to previous studies, hospitalization rates declined after the first year by about half with a stable rate over the next 5 years.

A meta-analysis of hospitalization rates in IBD was published from nine European countries based on the data of the national statistic offices in 2009^[37]. Hospitalization rates varied significantly among countries, ranging between 1.2 and 4.3 discharges per 10000 for CD. The highest rates were found in Denmark (4.33) and Scotland (4.15), with the lowest in Spain (1.20), Switzerland (1.31) and the Netherlands (1.46), a trend partly unrelated to disease prevalence. Numbers were similar for UC and CD in the given country with a specific age-distribution pattern (CD: High peak in 20-30 year old patients and small peak in the elderly; UC: Opposite trend).

Finally, multiple studies investigating US national databases reported an increase in CD related hospitalization rates. However, it is difficult to determine if this rise is associated with disease prevalence, severity or both. According to the National Hospital Discharge Survey database, CD-related hospitalization rates increased significantly from 9.3 to 17.1 per 100000 from 1990 to 2003^[38]. In particular, hospitalization rates in the 45-64 year-old and > 65 year-old groups rose significantly, while rates in younger patients remained essentially unchanged^[39]. Similar trends were reported from the Nationwide Inpatient Sample^[40]. Hospitalization rates increased 4.3% annually between 1998 and 2004. In contrast, data from Kaiser-Permanente suggested a decrease in CD-related hospitalization rates by about one-third between 1998 and 2005^[41] parallel with an increased use of IBD related drugs (including a fivefold increase in anti-TNF use) and a shift in gastroenterology-related visits from the gastroenterology division to primary care.

In conclusion, although hospitalization patterns and causes may have changed, rates are still high, with approximately 50% of CD patients requiring hospitalization within 10 years of diagnosis. Actual rates may vary significantly among age groups, time periods, reimbursement settings, and among countries. Findings must be interpreted with attention given to the context of disease prevalence, treatment strategy, and health care access.

SURGERY IN CROHN'S DISEASE: RATES, TRENDS AND CAUSES

Surgery is one of the most objective outcome measures, since it is only performed if clinically indicated. Almost decade ago, partly based upon historical data, the probability of surgery was reported between 3% and 96% within 15 years of diagnosis^[42], with clinical relapse and reoperation rates of 50%-60% and 28%-45%, respectively, during the subsequent 15 years. Surgical resection rates over time vary widely among published studies, ranging between 25% and 61% in the first five years. Early studies reported extraordinarily high surgical rates, as high as 30%, 50%, and 60% at 5, 10, and 15 years, respectively, in the population-based Stockholm County cohort from 1955-1974^[43]. Surgical rates did not seem to change according to an update from the same cohort^[44]. Even higher rates were reported some years later in a population-based cohort from Denmark^[45], with up to 35% of CD patients requiring surgery in first year after diagnosis. The cumulative surgery rate was 61% and 82% after 10 and 20 years.

Lower surgery rates were reported in the pre-biologic IBSEN cohort^[11]. In patients diagnosed between 1990-1994, surgery rates of 14%, 27%, and 38% at 1, 5, and 10 years were observed. Similar surgery rates were reported from the multinational European EC-IBD cohort diagnosed in the same time period with a cumulative surgery rate of 37.2% after 10 years and reoperation rates of 2.2%, 18.5%, and 35.9% at 1, 5, and 10 years, respectively^[51]. A geographic variability was reported. Patients from northern European centers, especially Copenhagen, had higher surgical need due partly to differences in disease phenotype. Interestingly, cumulative surgery rates were comparable from a recent publication from a referral center in South Korea^[46], which reported data from 1991 to 2007, which showed cumulative probability of surgery of 15.5%, 25.0%, and 32.8%, at 1, 5, and 10 years after diagnosis, respectively. Surgery rates in referral center may not be directly comparable with that reported from population-based studies, however. Geographic variability is also evident in Asia, as surgery rates were much higher in a Japanese referral center cohort^[47], reaching as high as 37.6%, 60.4%, and 74.2% at 5, 10, and 15 years. This is comparable to historical studies from Europe in the 1960s and may represent a distinct patient management strategy.

An association with disease phenotype was reported in multiple studies. Terminal ileal location, stricturing or penetrating disease, and younger age at diagnosis (< 40 years) were identified as risk factors for surgery. Recent data from Canada, Denmark, the United Kingdom, and Hungary, however, suggest that surgical rates were falling (Table 1) prior to the advent of biologic therapy, as summarized by the IOIBD Epidemiology Task Force report^[8]. This trend is best highlighted by a Danish study^[12]. The rate of early surgery (within one year of diagnosis) fell from 35% to 12% between 1962 and 2004. Risk has

Table 1 Surgery trends for Crohn's disease in population based cohorts by years from initial diagnosis

Geographic region and time period of investigation	Time from diagnosis		
	1 yr	5 yr	10 yr
North America/Asia			
Olmsted County, MN, United States ^[38]			
1970-2009		38%	48%
Manitoba, Canada ^[2]			
1988-2008	13%	24%	32%
2001-2008	10%	18%	
South Korea ^[39]			
1991-2007	15%	25%	33%
Europe			
Sweden ^[21]			
1955-1974		30%	50%
Denmark ^[25-37]			
1960-1978	35%		61%
2003-2005	12%		
Denmark ^[51]			
1979-1986		44.70%	
2003-2011		19.60%	
Norway ^[28]			
1990-1994	14%	27%	38%
Wales, United Kingdom ^[32]			
1986-1991	32%	59%	
1992-1997	25%	37%	
1998-2003	19%	25%	
Veszprem Province, Hungary ^[32,33]			
1977-2008	15%	31%	52%
2002-2006	10%	21%	
EC-IBD ^[29]			
1991-2003			40%

¹Referral cohort.

continued to decline, parallel with increased use of immunosuppressives and biologicals, although causality was not established^[48]. Similar trends were reported in a population-based CD cohort from Manitoba, Canada^[37,38]. Surgery rates at one and five years decreased from 13% and 22% in patients diagnosed between 1996 and 2000 to 10% and 18% for those diagnosed between 2001 and 2008 (HR = 0.79; 95%CI: 0.65-0.97). Reoperation rates were unaffected by the era of diagnosis. In contrast, high operation rates were reported from the Mayo Clinic^[10] in patients diagnosed between 1940 and 2001 with a cumulative risk for surgery 24%, 49%, and 64% at 1, 10 and 30 years from diagnosis, respectively. In an update of the same cohort, presented in an abstract form, surgery rates did not seem to decline in patients diagnosed between 1970 and 2004 with 38%, 48%, and 61% of patients being operated on at 5, 10, and 30 years.

An association was also suggested with a change in disease management including tight follow-up and early immunomodulator therapy, however data are partly conflicting. In a previous referral center study from France, the need for intestinal surgery did not decrease despite increased use of immunosuppressants^[49]. However, in this study immunosuppressives were almost exclusively started after surgery. In contrast, recent population-based reports from Wales and Hungary^[50,51] reported that early azathioprine (AZA) use may be associated with reduced

frequency of resective surgery. In the study from Wales, surgery rates decreased from 59% to 25% at five years after diagnosis between 1986 and 2003. A similar five-year surgery rate (21.3%) was reported in the latter study in patients diagnosed between 2002 and 2006^[13]. In addition, a French study reported an association between the duration of anti-TNF and AZA therapy and risk for surgery^[52]. Of course, long treatment duration allows responders to the above therapies to be identified.

While data are mixed and there exists geographic variation, recent data suggest a multifactorial trend for decreasing surgery. Disease behavior at diagnosis as reported in the most recent studies is more often inflammatory compared to earlier CD cohorts^[15,16,18]. In addition, diagnostic tools and follow-up strategy has changed significantly in the last decade, parallel with the earlier and more widespread use of immunosuppressives, as reported in a recent publication from Canada^[37]. In this study, authors reported an association between early gastroenterologist care and lower risk of surgery parallel with an increased early use of immunosuppressives. However, exposure to immunosuppressives is still relatively limited in the population-based studies and reoperation rates are essentially unchanged.

However, results from two recent prospective randomized clinical trials cast some doubt on the efficacy of early thiopurine therapy. In the first paper, the GETAID group^[53] reported that early aggressive therapy with AZA (2.5 mg/kg) within 6 mo of diagnosis was no more effective than conventional management in increasing time of clinical remission as assessed by trimesters for 36 mo. However, 61% of the patients in the “conventional” group required AZA within a median of 11 mo of diagnosis, which cannot be interpreted as a conservative approach. Therefore, a more accurate interpretation is that authors compared early aggressive strategy with an early-accelerated strategy, and still the need for perianal surgery was lower (4% *vs* 18%, $P = 0.036$). Another study, AZ-TEC, from the Spanish IBD group^[54], appears promising in design; early CD patients (< 8 wk of diagnosis), after entering remission, patients were randomized to receive AZA or placebo. The endpoint was steroid-free remission at week 76. Unfortunately, the trial was stopped for futility; therefore the power of the study is somewhat questionable.

A more precise interpretation of the results reveals difficulties. First, diagnosis can still change in approximately 10% to 15% of CD patients during follow-up, as suggested the IBSEN group. Thus, 8 wk from the first specialists visit and diagnosis may introduce some unintentional bias with regards to the above. Second, we must assume that 30% of patients entered remission without steroid therapy, since under standard steroid taper schedules patients treated with steroids at diagnosis should still have received steroids at 8 wk. In addition, approximately one fourth of patients entered the trial without clinical remission. In contrast, median C-reactive protein (CRP) was low (CRP at diagnosis was not given). Of note, 92%

of patients had inflammatory disease, extensive location was observed in only one-third of patients, and patients with fistulizing (internal penetrating or perianal fistula) or stenosis were excluded. Thus we propose an alternative interpretation of the findings: mild phenotype patients at diagnosis do not necessarily benefit from early AZA therapy in the short term. However, this trial does not provide data on the efficacy of early AZA therapy in patients with complicated disease phenotype at diagnosis, nor whether AZA has the potential to change the natural history of the disease. In addition, the definition of clinical relapse was based simply on CD activity index (CDAI) and this does not adequately define steroid-free status, since under this definition most patients would have a relapse as defined by a CDAI elevation before they would need steroids. This is indeed a very soft endpoint. Interestingly, with a modified definition of relapse (CDAI > 220) AZA patients had a significant clinical benefit, even bearing in mind the limitations of CDAI. From this trial, it should be clear that the use of CDAI is insufficient as the only definition of relapse. Other objective parameters are needed, such as a change in CDAI > 100 from baseline, a need for a change in the medical therapy, or the development of complications. Development of complicated disease or need for surgery would be the optimal outcome measures to study the natural history of the disease.

Of note, surgery should not always be regarded as a negative outcome, and it has an important place in the management of CD patients. Early surgery has been shown to prolong clinical remission (HR = 0.57; 95%CI: 0.35-0.92)^[55]. In addition, CD patients with limited complicated terminal ileitis diagnosed at surgery were reported to have low reoperation rates, and needed less steroids and immunosuppressants during follow-up than those not diagnosed intra-operatively^[56]. The same was proven for early terminal ileum resection in a population-based Hungarian cohort^[57]. In these patients, surgery is part of a proactive treatment strategy and possibly represents an alternative to medical therapy. On the other hand, surgery during the first 6-10 mo of diagnosis is clearly linked to unavoidable complications already present at diagnosis. Unfortunately, this is more representative of the initial cohort characteristics and should not be interpreted as a real outcome measure. Thus, if we would like to study the association between management and treatment strategy most probably these patients should be excluded from the analysis. Finally, the above surgery rates and trends were reported from the pre-biologic era in cohorts with no or only minimal or anti-TNF/biological exposure. Whether biological therapy directly influences long-term surgery trends outside of clinical trials remains unclear.

MORTALITY

In a meta-analysis from 2010, mortality in CD was increased with a pooled standardised mortality (SMR) of 1.39 (95%CI: 1.30-1.49)^[58]. The meta-analysis included

Table 2 Key issues on the natural history of Crohn's disease

- The distribution of location in Crohn's disease (CD) has not changed significantly in the recent decade, but differs according to age at onset
- Recent data indicate that there are an increasing proportion of Crohn's disease patients are diagnosed with an inflammatory disease behavior. The progression to complicated disease phenotype is decreased
- There is evidence from population-based studies that the surgery rates have recently declined in Crohn's disease
- Data suggest that the decline in the surgical rates is partly associated with early use of thiopurines. However, the relative importance of changes in treatment strategy and patient monitoring on the natural history remain conflictive
- Overall mortality rates in CD have been higher than that in the background population, and there is only little evidence that these have changed in the last decade. In addition, an increased mortality from gastrointestinal causes is constantly reported
- Further data are needed to assess whether tight, and objective patient monitoring (including clinical, laboratory, endoscopy and imaging) or early administration of biological would lead to superior outcomes
- Cost-effectivity of the new treatment and monitoring strategies has to be established

nine population-based studies of which eight were European (including an EC-IBD study). Causes identified were cancer, COPD, gastrointestinal disease, and genitourinary disease. A recent nationwide study from Denmark confirmed a 50% increased mortality in CD, and concluded that mortality in CD did not decrease over time, despite a change in patient management^[59]. Similar results were published some years earlier in another meta-analysis^[60], which included referral center data. In subgroup analyses, the SMR ratio was increased in hospitals (SMR = 1.73; 95%CI: 1.45-2.47), referral centers (SMR = 2.06; 95%CI: 1.63-2.60), and population-based studies (SMR = 1.48; 95%CI: 1.28-1.70).

In contrast, the authors of two very recent population-based studies failed to confirm an increase in the overall CD mortality. In a study from Finland, mortality was not increased in 1915 adult IBD patients in 1986-2007. Mortality was increased from diseases of the digestive system, but there was a reduced mortality from mental and alcohol-related behavioral disorders compared to the general Finnish population^[61]. Another recent population-based study from South-Limburg, in the Netherlands did not find increased overall mortality in CD between 1991 and 2003 (SMR = 1.1; 95%CI: 0.7-1.6), despite increased mortality from gastrointestinal causes (SMR = 7.5; 95%CI: 2.8-16.4) in this patient group^[62]. This concurs with previous reports from the Mayo Clinic, where authors did not find increased mortality in 314 patients between 1940-2001 (SMR = 1.2; 95%CI: 0.9-1.6)^[63]. In addition, an increased risk of dying from non-malignant gastrointestinal causes (SMR = 6.4; 95%CI: 3.2-11.5), gastrointestinal malignancies (SMR = 4.7; 95%CI: 1.7-10.2), and COPD (SMR = 3.5; 95%CI: 1.3-7.5) was also observed. In contrast, another study from Kaiser Permanente reported increased mortality in CD patients between 1996 and 2003 (SMR = 1.4; 95%CI: 1.2-1.6)^[64]. In conclusion, there is insufficient evidence to support the hypothesis that overall CD mortality trends

has changed, it is slightly increased together with a consistently increased mortality having been reported from gastrointestinal causes.

SUMMARY AND CONCLUSION: IS THE NATURAL HISTORY OF CD CHANGING?

Studies on the natural history of CD provide invaluable data on the disease course as well as clinical predictors, and may help identify patient subsets based on clinical phenotype. Most data are available from referral centers, however outcomes are different from data reported from population-based cohorts, so that results are not directly comparable.

New data suggest a possible change in the natural history of Crohn's disease (Table 2), with increasing numbers of patients diagnosed with inflammatory disease behavior, likely, one would hope, due to new diagnostic techniques and tools. Hospitalization rates remain high, yet hospitalization is a relatively soft endpoint, and actual rates may vary significantly according to age group, reimbursement setting, and countries. Findings must be interpreted with attention to disease prevalence, treatment strategy, and health care access. In contrast, surgery rates seem to have decreased in the last decade, yet it is difficult to identify the drivers of this change. A combination of the greater proportion of patients with uncomplicated disease behavior, changes in patient monitoring, different therapeutic strategies, and altered attitude towards surgery may be at least partly responsible. Finally, mortality rate in CD still exceeds that in the general population and there is only little evidence that this has changed.

The impact of changing treatment strategy on the above trends, including increased, earlier use of immunosuppressives and biologicals, and changed systems for patient monitoring on the natural history is not entirely clear. Unfortunately, data from randomized clinical trials are of limited value in studying the natural history of the disease. This is partly because follow-up is limited in duration and open-label extensions include the same confounders as population-based cohorts. In addition, the patient populations do not reflect the patients from everyday clinical practice, as highlighted by a recent paper from the United States^[65]. Therefore, a direct extrapolation of the findings to the clinic is often difficult.

In conclusion, for clinical practice, it is important to use available results from the published literature. We must identify markers of progressive as well as mild disease, since an early patient stratification enables clinicians to select the most appropriate therapy for a given patient. Further data are needed to investigate whether tight, objective patient monitoring and early administration of biological agents lead to superior outcomes. Some clinical trials are underway (CALM, REACT) and results will be available soon. However, the cost-effectiveness of the new treatment and monitoring strategies also must be established in the near future. In addition, it will be extremely important to follow-up the recent multinational,

multicenter, population-based patient cohorts (EpiCom and ACCESS), since accurate long-term data on harder endpoints such as complications, surgery, and ultimately mortality in the biological era is urgently awaited, and can be obtained only in this setting. The key factor is the appropriate adjustment for confounders.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Interleukin and interleukin receptor gene polymorphisms in inflammatory bowel diseases susceptibility

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systematic review of the current literature on interleukin and interleukin receptor gene polymorphisms associated with IBD, performing an electronic search of PubMed Database from publications of the last 10 years, and used the following medical subject heading terms and/or text words: IBD, CD, UC, interleukins and polymorphisms.

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Interleukin; Interleukin receptor; Polymorphisms

Core tip: Inflammatory bowel diseases (Crohn's disease and ulcerative colitis) are chronic, progressive disorders of the gastrointestinal tract. Different genes, including interleukin genes play central role in mediating and modulating of inflammation in inflammatory bowel diseases. In this review we summarized the interleukin and the interleukin receptor genes associated with Crohn's disease and/or ulcerative colitis performing an electronic search on the PubMed database focusing on the following terminology: inflammatory bowel disease, Crohn's disease, ulcerative colitis, interleukin and interleukin receptor.

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Abstract

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), represents a group of chronic inflammatory disorders caused by dysregulated immune responses in genetically predisposed individuals. Genetic markers are associated with disease phenotype and long-term evolution, but their value in everyday clinical practice is limited at the moment. IBD has a clear immunological background and interleukins play key role in the process. Almost 130 original papers were revised including meta-analysis. It is clear these data are very important for understanding the base of the disease, especially in terms of clinical utility and validity, but text often do not available for the doctors use these in the clinical practice nowadays. We conducted a

INTRODUCTION

Inflammatory bowel disease (IBD) - clinically classified as Crohn's disease (CD; OMIM 26600) and ulcerative

colitis (UC; OMIM 191390) - is a common chronic, relapsing inflammatory disorder of the gastrointestinal tract^[1]. In Europe the highest annual incidence of CD is 12.7/100000 and 24.3/100000 for UC. In Asia and in the Middle East both rates are much lower (CD: 5.0/100000 and UC: 6.3/100000). However in North America the incidence for UC is 19.2/100000 and they have the highest rate for CD in the world with 20.2/100000^[2]. Although the precise etiology of IBD still remains obscure, the accepted hypothesis is that in genetically predisposed individuals the commensal luminal flora trigger an inappropriate, overactive mucosal immune response causing intestinal tissue damage that is further modified by specific environmental factors (*e.g.*, smoking)^[3].

The location of inflammatory lesions and the types of cytokines involved in the pathogenesis mainly distinguish CD from UC. Whereas CD is a segmental, transmural disorder involving any part of the gastrointestinal tract, UC is characterized by superficial, continuous mucosal ulcers restricted to the colon. Imbalances between pro- and anti-inflammatory cytokines in the mucosa have been established for both CD and UC^[4]. CD is associated with a T helper type 1 (Th1)^[4] and T helper type 17 (Th17)^[5] immune response, thus interferon gamma/interleukin 12 (IFN γ /IL12) and IL23/IL17 cytokines assign the downstream release of complex network of further pro-inflammatory cytokines (*e.g.*, IL18, IL2, IL1, IL21, IL22) (Figure 1). Th17 and a modified Th2 cytokine profile (IL13 and IL5) are characteristic for UC. In addition, IL6 and tumor necrosis alpha (TNF α) are produced by both Th1 and Th2 cells as well as by macrophages in both IBD entities. A further group of T cells, regulatory T cells (Treg) cells are important for the control of immune responses to self-antigens preventing autoimmunity and maintaining self-tolerance^[6]. The final result of this activated cytokine network is the recruitment of more effector cells and the beginning of mucosal inflammation, which will eventually become chronic due to defective regulation of the immune response^[6].

First, genome-wide association studies (GWAS) resulted in the identification of many novel susceptibility loci CD and later for UC^[7,8]. To date, the number of known risk loci has expanded to 163^[9]. Some loci seem to be specific either to CD or to UC, whereas others confer common susceptibility to IBD; approximately 30% of IBD-related genetic loci are shared^[10,11]. The IBD-associated loci encode genes involved in innate pattern recognition [nucleotide-binding oligomerization domain-containing protein 2 (NOD2), autophagy (autophagy-related protein 16-1 (ATG16L1), immunity-related GTPase family M protein (IRGM), differentiation of Th17-T lymphocytes (IL23R), maintenance of epithelial barrier integrity IBD5 locus], and coordination of adaptive immune responses [human leukocyte antigen (HLA)-region]^[12]. Polymorphisms in genes encoding cytokines and cytokine receptors may affect the course of the inflammatory cascade and thereby increase the risk of developing IBD.

In this review we discuss in each of the IL families

only those interleukins or interleukin receptors in detail, which have relevant polymorphisms in IBD, CD or UC (Figure 2).

SYSTEMATIC REVIEW

We conducted a systematic review of the literature of the last 10 years on interleukin susceptibility genes to IBD. PubMed was searched for papers and abstracts published in English-language journals. We used the following medical subject heading terms and/or text words: “inflammatory bowel disease”, “ulcerative colitis”, “Crohn’s disease” and “cytokines”. The search was focused on interleukin susceptibility genes polymorphism resulting in IBD. No restrictions were placed on race, ethnicity, or geographic area. Extraction from each study was conducted independently by all authors, and consensus was achieved for all data.

INTERLEUKIN AND INTERLEUKIN RECEPTOR GENE POLYMORPHISMS

ILs are the subset of a larger group of cellular messenger molecules called cytokines, which are humoral, small (4-15 kDa) inducible immune-regulatory proteins or glycoproteins which mediate communication between cells, regulate cell growth and differentiation, and play a central role in the development and homeostasis of the immune system^[6]. They act on target cells by binding to specific IL receptors, initiating signal transduction and second messenger pathways within the target cell. This can result in gene activation, lead to mitotic division, growth and differentiation, migration, or apoptosis. Cytokines act in a highly complex coordinated network in which they induce or repress their own synthesis as well as that of other cytokines and cytokine receptors. The nomenclature of ILs is continuously evolving (www.genenames.org/genefamilies/IL); they are assigned to each family based on sequence homology and receptor chain similarities or functional properties (Table 1)^[13].

IL1 FAMILY

The IL1 family is a group of 11 cytokines (IL1A, IL1B, IL1RN, IL18, IL33, IL36A, IL36B, IL36G, IL36RN, IL37 and IL38), which have similar gene structure and induce a complex network of proinflammatory cytokines. The interleukin 1 receptor (IL1R) family also expands to 9 distinct genes and includes coreceptors, decoy receptors, binding proteins, and inhibitory receptors^[14].

IL1

IL1 is a potent proinflammatory cytokine, which affects cell proliferation, differentiation, and the function of many innate and specific immunocompetent cells, and acts as an endogenous pyrogen. It also mediates many inflammatory diseases by initiating and potentiating im-

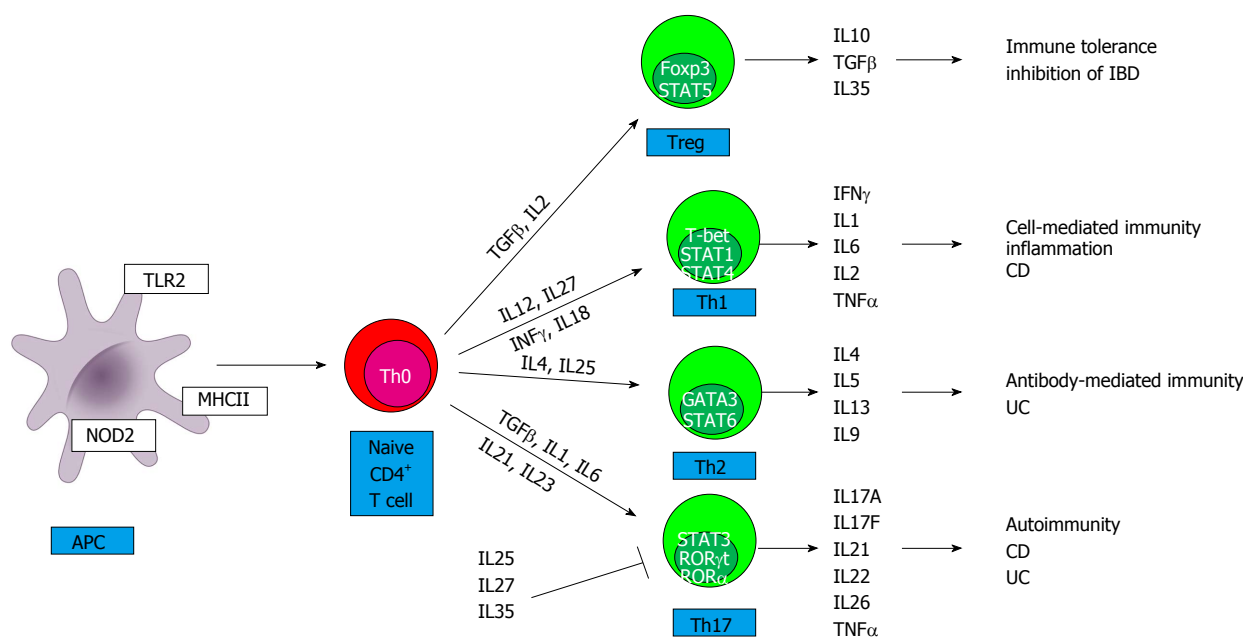


Figure 1 Differentiation of effector T helper and regulatory T cells in inflammatory bowel disease. Antigen presenting cells (APCs) (*i.e.*, macrophages and dendritic cells) in the lamina propria are increased in absolute number in both forms of inflammatory bowel disease (IBD). First, microbial products (pathogen associated molecular patterns, PAMPs) bind to a group of detection molecules of the innate immune system, called pattern recognition receptors (PRRs). This includes Toll like receptors (TLRs) on cell surface intracellular compartments, and the cytoplasmatic nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors (NLR) family. Stimulation of these receptors induces intracellular signaling cascades, resulting in secretion of large number of cytokines, chemokines, and immuno-modulatory factors. APCs interact with T cells by presenting an antigen on the surface of the major histocompatibility complex II (MHCII), which is recognized by the appropriate T cell receptor. The development of T helper (Th)1, Th2, Th17 and regulatory T cells (Treg) subsets from naive, Th0 cells during primary immune response is mainly determined by this cytokines and chemokines. It is under the control of certain transcription factors: T-box expressed in T cells (T-bet), GATA binding protein (GATA3), retinoid-related orphan receptor (RORγt), RORα, signal transducer and activator of transcription (STATs) and forkhead box P3 (FoxP3). Interleukin (IL)12 is the hallmark cytokine for Th1 cell lines, which produce interferon gamma (IFNγ) and are important for host defense to intracellular pathogens. IL4 promotes differentiation into Th2 cells, which produce IL4, IL5, and IL13 and participate in controlling humoral immunity to extracellular parasites and allergic inflammatory responses. Th17 cells develop from naive T cells in the presence of transforming growth factor beta (TGFβ), IL23, IL1B or IL6. The effector cytokines IL17A, IL17F, and IL22 play key roles in Crohn's disease (CD) and ulcerative colitis (UC), and in other autoimmune diseases. TGFβ and IL2 together convert naive T cells into regulatory T cells, which promote self tolerance and prevent autoimmunity. CD is a predominantly Th1 and Th17-mediated disorder, while UC is associated with a Th17 and a modified Th2 cytokine profile.

immune and inflammatory responses^[13].

IL1 is made up of two major proteins: IL1A (OMIM 147760) and IL1B (OMIM 147720)^[15]. These proteins exert similar effects, first, by binding to the first extracellular chain of the IL1 type I receptor (IL1RI) (OMIM 147810) that recruits the IL1 receptor accessory protein (IL1RAP) (OMIM 602626), which serves as a coreceptor and is necessary for signal transduction. IL1A and IL1B are also able to bind to the IL1 type II receptor (OMIM 147811), which acts as a decoy receptor and is not involved in signal transduction^[13].

IL1B has an important role in initiating and amplifying the inflammatory response^[16]. Normal colonic mucosa cells produce very little mature IL1B, however in the mucosa of affected IBD patients, a large amount of mature IL1B is produced^[17,18]. The inability of normal intestinal macrophages to produce mature IL1B could result from regulation at one or more steps from gene activation to post-translational processing of the propeptide by IL1B converting enzyme and release of the mature peptide^[19,20].

The IL1 receptor antagonist (IL1RN) (OMIM 147679) is an anti-inflammatory cytokine, which is synthesized and released in response to the same stimuli that lead to

IL1 production^[21]. IL1RN lacks the IL1RAP interacting domain, so that binding of the IL1RN to IL1RI inhibits IL1 signaling^[15]. In IBD and several other inflammatory diseases, an imbalance the IL1RN/IL1 ratio contributes to the chronic inflammatory response^[22-24]. Polymorphisms of the *IL1RN* gene, which can lead to changes in the IL1RN and IL1 balance, are associated with susceptibility to UC^[25]. Moreover, it is well accepted that IBD patients have a decreased ratio of IL1RN/IL1B in their colonic mucosal tissue^[26].

The variant alleles of two IL1B promoter polymorphisms, IL1B T-31C and IL1B C-511T, have been found to be in almost complete linkage disequilibrium, and the haplotypes encompassing the IL1B T-31C variant conferred higher transcription of IL1B compared to the wild type haplotype^[27].

Four polymorphisms (rs315951, rs315952, rs419598 and rs16944) in the *IL1B* and *IL1RN* genes were analyzed in Mexican Mestizo UC patients. The first 3 single nucleotide polymorphisms (SNPs) are located in the *IL1RN* and the fourth one in the *IL1B* gene. The first two (rs315951 and rs315952) are associated with the risk of developing UC. They found significant increased frequencies of IL1RN6/1TC (rs315952) and RN6/2CC

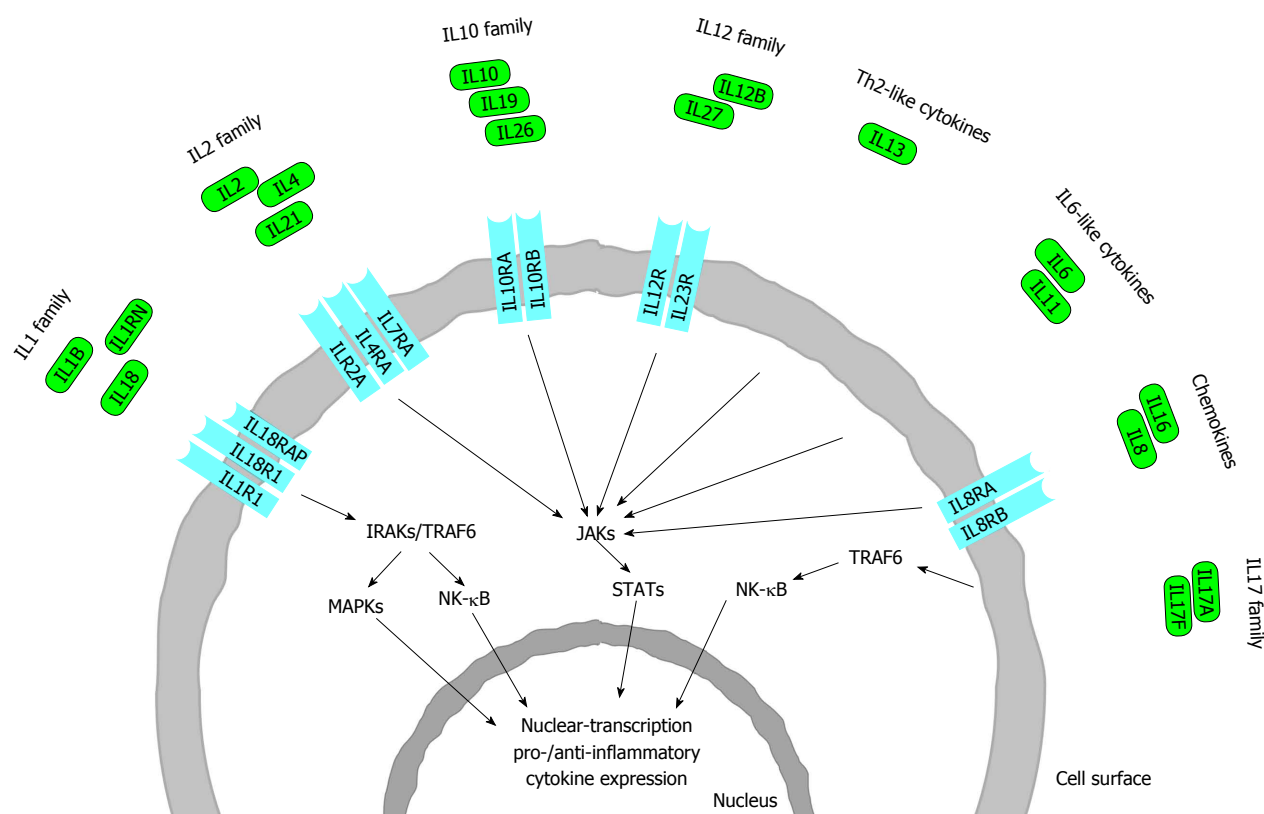


Figure 2 Schematic representation of the interleukin families and receptors involved in the pathogenesis of inflammatory bowel disease. Only those interleukins (IL) and IL receptors (ILR) are shown where studies have demonstrated association between genes/single nucleotide polymorphisms (SNPs) and disease phenotype. ILs are assigned to each family based on sequence homology and receptor chain similarities or functional properties, considerable overlap between these families exists. Polymorphisms in genes encoding ILs and ILRs have been found to be involved in inflammatory bowel disease. Ligand binding initiates intracellular phosphorylation cascades that are mediated by kinases (*i.e.*, IL1 receptor associated kinase (IRAK); mitogen-activated protein kinase (MAPK); Janus kinase (JAK) and TNF receptor associated factor (TRAF), resulting in signal transduction through certain transcription factors [including signal transducers and activators of transcription (STAT); nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)]. These transcription factors stimulate the expression of a number of proinflammatory and anti-inflammatory cytokine genes involved in inflammatory bowel disease (IBD).

(rs315951), and decreased frequency of IL1B-511 TC (rs16944) genotypes in UC patients. UC patients showed increased frequencies of IL1RN CTC and TCG haplotypes, whereas TTG and CTG haplotypes showed decreased frequency in UC patients. They also found decreased gene expression of IL1RN level in the mucosa from UC patients carrying the rs315951 GG genotype when compared with UC patients with the rs315951 CC genotype^[28].

IL18

One of the main function of IL18 (OMIM 600953) is to promote the production of IFN γ from T and natural killer (NK) cells, particularly in the presence of IL12p70. First it binds to its ligand binding chain the interleukin 18 receptor 1 (OMIM 604494), recruits its coreceptor the IL18 receptor accessory protein (IL18RAP) (OMIM 604509), and the activation of nuclear factor kappa-light-chain-enhancer of activated B cells/mitogen activated protein 8 is initiated. IL18 expression correlates with the activities of CD^[29].

IL18 binding protein (IL18BP, OMIM 604113) is able to prevent the binding of IL18 to its receptor, and

thereby blocks its downstream functional effects. IL18BP has neutralizing isoforms, which have increased levels in the intestinal tissue of active CD patients^[30].

Several polymorphisms were studied in the *IL18* gene: the A105C, the T113G and the C127T in the coding region, and the G-137C, the C-607A and the G-656T in the promoter region. In the Japanese population significant difference was found in the allele frequency of A105C between CD patients and healthy controls. However, there was no association between A105C and UC^[31]. In another Japanese study the G allele at 113 and the T allele at 127 were significantly higher in patients with IBD compared to the control^[32]. In the third Japanese study allele and genotype frequency of G-137C were significantly higher in the proctitis-type UC patients than in controls^[33]. The frequency of haplotype 2 (-607A, -137C), which have lower promoter activity and IFN γ - mRNA level was significantly increased in the proctitis-type patients than in the control group^[33]. Any significant differences in allele or genotype frequencies were observed in the CD group^[33]. The C-607A and the G-137C SNPs in the promoter region were associated with the development of UC but not with CD in Tunisian patients. The

Table 1 Characteristics of cytokines in inflammatory bowel diseases

Family	Cytokine	Receptor	Cytogenetic location	Molecular weight	Cell source	Disease association (IBD)
IL1	IL1A (IL1F1)	IL1R1	2q14	17 kD	Macrophages, monocytes, lymphocytes, keratinocytes, microglia, megakaryocytes, neutrophils, fibroblasts and synovial lining cells	CD, UC
	IL1B (IL1F2)	IL1R1 IL1R2	2q14	17 kD		CD, UC
	IL1RN (IL1F3)	IL1R1 IL1R2	2q14.2	16.1-20 kD	Monocytes, macrophages, fibroblasts, neutrophils, epithelial cells and keratinocytes	UC
	IL18 (IL1F4)	IL18R1 IL18RAP	11q22.2-q22.3	22.3 kD	Macrophages, Kupffer cells, keratinocytes, osteoblasts, astrocytes, and DCs	CD, UC
IL2	IL2	IL2R	4q26-q27	15.5 kD	CD4+, CD8+ activated T cells, DCs, NK and NKT cells	CD, UC
	IL4	IL4R I IL4R II	5q23-q31	15 kD	Th2 cells, basophils, eosinophils, mast cells, NKT and γ/δ T cells	CD
	IL21	IL21R	4q26-q27	15 kD	T and NKT cells	CD, UC
IL10	IL10	IL10RA/IL10RB	1q31-q32	18.6 kD	T and B cells, monocytes, macrophages and DCs	CD, UC
	IL19	IL20RA/IL20RB	1q32.2	35-40 kD	Monocytes, keratinocytes, airway epithelial cells and B cells	UC
IL12	IL26	IL10R2/IL20R1	12q15	38 kD	Activated T cells	CD, UC
	IL12	IL12RB1/IL12RB2	5q33.3	IL12A: 35 kD, IL12B: 40 kD	Monocytes, macrophages, neutrophils, microglia, DCs and B cells	CD, UC
	IL23	IL12RB1/IL23R	12q13.13	19 kD	Macrophages and activated DCs	CD, UC
	IL27	IL27RA/ IL6ST	16p11	IL27A: 2.8 kD IL27B: 25.4 kD	Activated DCs, macrophages, and epithelial cells	CD, UC
IL6-like cytokines	IL6	IL6R/IL6ST	7p21-p15	19-26 kD	Endothelial cells, fibroblasts, monocytes/macrophages	CD
IL17	IL17A	IL17RA/ IL17RC	6p12	35 kD	Th17, CD8+ T cells, NK cells, NKT cells, γ/δ T cells and neutrophils	UC
	IL17F	IL17RA/IL17RC	6p12	44 kD	Th17, CD8+ T cells, NK cells, NKT cells, γ/δ T cells and neutrophils	CD, UC
Chemokines	IL8	IL8RA/IL8RB	4q13-q21	16 kD	Monocytes, macrophages, neutrophils, lymphocytes, endothelial cells, epithelial cells, fibroblasts, keratinocytes, chondrocytes, synovial cells, and hepatocytes	CD, UC
	IL16	CD4	15q26.3	56 kD	T cells, eosinophils, mast cells, eosinophils, monocytes, DCs, fibroblasts and epithelial cells	CD
Th2-like cytokines	IL13	IL13RA1/IL13RA2	5q31	10 kD	T, NKT, mast cells, basophils and eosinophils	CD, UC

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; NK: Natural killer cells; NKT: Natural killer T cells; DCs: Dendritic cells.

-137GG genotype frequency was significantly higher in UC than in controls. Statistically significant association was found between -607AA genotype in UC patients and the distal localization of the lesions^[34]. However the polymorphism G-137C was not found a susceptibility factor for IBD in a German population^[35].

Recent GWAS study^[36] and meta-analysis confirmed the *IL18RAP* region as CD locus^[8]. The rs6708413 G allele is a shared risk locus for CD and Celiac disease^[37]. In individuals homozygous for the risk allele, the genotypes strongly correlate with lower IL18RAP expression which may lead to differential IL18-mediated innate immune responses to infection^[38]. Strong association of rs917997 SNP was demonstrated for both CD and UC^[39]. In a new GWAS study association of CD and IBD with coding variant V527L was found. This rare missense increased

high the risk for CD^[40].

IL2 FAMILY

The IL2 family consists of IL2, IL4, IL7, IL9, IL15 and IL21. This family of cytokines encompasses a group of interleukins which share a common receptor subunit, the "common γ chain", which acts in unison with a subtype specific α -chain to initiate the signaling cascade. This ILs act mainly as growth and proliferation factors for progenitors and mature cells and also have roles in lineage-specific cell differentiation^[13].

IL2

IL2 acts as a T cell growth factor and promotes proliferation and differentiation of NK cells to increase their cy-

tolytic functions. IL2 is essential for the development of Th1, Th2, Treg, and Th17 differentiation^[41].

The IL2 receptor (IL2R) consists of three non-covalently associated proteins: IL2RA (OMIM 147730), IL2RB (OMIM 146710) and IL2RG (OMIM 308380). The α -chain is produced when the T cell is activated by antigen and constitutes the high affinity receptor together with the other two subunits^[42]. The β - and γ -chains form the intermediate affinity IL2 receptor^[43].

Several SNPs (rs6822844, rs13151961, rs13119723 and rs6840978) in the *IL2/IL21* block were analyzed in different populations. In a Dutch cohort the minor alleles of these SNPs were associated with IBD. In UC patients the effect was even stronger. However in the CD subgroup, the rs13119723 SNP was only borderline significant, while only a trend towards association was found for the other SNPs. Testing of all four SNPs in the Italian cohort, the same strong association of the minor alleles in UC was found as in the Dutch cohort. The CD subgroup of the Italian cohort showed only a trend towards association with the same alleles. However in the Jewish population there was any significant association between any of the SNPs and CD. Similarly a North American study showed that these alleles have an influence on IBD. The effect was strongest in the UC subgroup likewise. In the CD subgroup of the North American cohort moderate association with the same alleles was also observed^[44].

IL4

IL4 (OMIM 147780), a pleiotropic cytokine, is the major stimulus of Th2-cell development, which regulates allergic conditions and the protective immune response against helminthes and other extracellular parasites^[45]. There are 2 types of IL4Rs. IL4R Type I binds only IL4 and consists of 2 receptor chains: IL4RA (OMIM 147781) and the common γ c, IL4R Type II binds IL4 and IL13 and consists of the IL4RA and the IL13RA1 chains^[46].

Functional polymorphism in the *IL4* gene promoter C-34T was associated with CD in a British population^[47]. The same polymorphism was tested in a New Zealand population, where no significant difference was observed in the genotype frequencies of controls vs CD patients^[48].

IL21

IL21 (OMIM 605384) is a cytokine with potent regulatory effects on cells of the immune system, including NK cells and cytotoxic T cells, which can destroy virus infected and cancerous cells^[49]. In contrast with its anti-cancer effects, IL21 also contributes to inflammation in several disorders, as can be expected for a Th17-related cytokine. The functional receptor of IL21 consists of γ c and the IL21RA (OMIM 605383)^[13].

GWAS provides evidence for 4q27 region *IL2/IL21* association with UC^[44] and CD^[50]. This region contains four genes in strong linkage disequilibrium: *KLA41109-TENR-IL2-IL21*.

IL10 FAMILY

The members of the IL10 cytokine family (IL10, IL19, IL20, IL22, IL24, IL26, IL28, and IL29) are mainly linked through their similar intron-exon structure. This family can be divided into viral and cellular homologs, where this last named group contains the above mentioned ILs^[51].

IL10

IL10 (OMIM 124092) is an anti-inflammatory cytokine, which is produced by monocytes, T cells, B cells, NK cells, macrophages, and dendritic cells. It inhibits both antigen presentation and subsequent release of pro-inflammatory cytokines. Thereby attenuates the activated immune system. The *IL10* gene maps to a cytokine cluster that includes *IL19*, *IL20*, *IL24*, and *IL6* genes. Two IL10RA (OMIM 146933) and two IL10RB (OMIM 123889) chains forms the heterotetrameric IL10 receptor complex. The IL10RB chain is shared with other cytokine receptors^[52].

In a GWAS the rs3024505 showed the most significant association in the combined verification UC samples. Association between rs3024505 and CD was weak. These results suggest that defective IL10 function is central to the pathogenesis of the UC subtype of IBD^[53]. In a latest study from 29 SNPs conferring high genetic susceptibility to CD, the rs3024505 of the *IL10* gene was associated with susceptibility to UC in Australian population^[54]. Similarly to this, the rs3024505 was associated with the risk of UC and CD in a Danish study^[55].

The *IL10* promoter polymorphisms G-1082A, C-819T, and C-592A have been most extensively studied. They are in tight linkage disequilibrium^[56]. Studies on the *IL10* promoter polymorphisms and IBD susceptibility have been controversial^[57-60]. In Spanish population the G-1082A and the C-819T polymorphisms in the *IL10* gene contribute to susceptibility to CD^[61].

In IBD patients in Tunisia the polymorphisms A-627C and G-1117A were analyzed and found as potential factors influencing IBD susceptibility and phenotype. However, no significant variations in genotypes frequencies were found comparing the CD and UC patients^[62].

The A-1082G variant was analyzed in Caucasian population in many studies. It was suggested that G carriers were more susceptible to UC^[63], whereas in another study G carriers were associated with lower UC incidence^[64]. Only two datasets concerning the relationship between A-1082G polymorphism and UC susceptibility in Asian subjects^[65,66] were identified. A meta-analysis showed no association between A-1082G polymorphism and UC susceptibility under any genetic models in overall analysis or in subgroup analysis^[67]. In another study this variant was significantly associated with the colonic localization of the disease in Caucasian CD patients (children) with French-Canadian origin^[68].

IL19

IL19 (OMIM 605687) might promote Th2-cell re-

sponses, because it induces expression of IL4, IL5, IL10, and IL13 by activated T-cells^[69]. IL19 functions as a monomer, binds to a heterodimeric receptor made up of IL20RA (OMIM 605620) and IL20RB (OMIM 605621). This complex also binds to IL20 and IL24^[70].

In GWAS, 14 previously identified UC susceptibility loci were analyzed. Association including the polymorphism rs3024505 in the *IL19* was confirmed^[71].

IL26

Expression of IL26 (OMIM 605679) seems to be restricted to memory T cells, NK cells, and Th17 cells. Thereby it could have proinflammatory effects in CD^[72]. The receptor for IL26 consists of IL10RB and IL20RA chains. Dambacher reported expression of both IL26 receptor subunits IL20RA and IL10RB by several intestinal epithelial cells (IEC) lines in CD^[73].

First time, the rs2870946-G and the rs1558744-A association were described with UC^[74]. Further meta-analysis study confirmed the association of rs1558744-A with UC^[71].

IL12 FAMILY

The IL12 family of cytokines includes IL12, IL23, IL27, IL30 and IL35, which are important mediators of inflammatory diseases. Each member is heterodimeric complex composed of two subunits whose expression is regulated independently and have very different biological activities^[75].

IL12

IL12 (also known as IL12p70) was first described as a NK stimulating factor. It mediates development and maintenance of Th1 cells by inducing production of IFN γ by Th1 and NK cells. IL12 indirectly activates the antimicrobial, antiparasitic, and antitumor activity of macrophages and promotes cytolytic activity of NK cells and lymphokine-activated killer cells^[76]. Reduced production of IL12 impairs Th1 responses and increases susceptibility to infection with intracellular pathogens. IL12 consist of p35 (IL12A, OMIM 161560) and p40 (IL12B, OMIM 161561) subunits, which is shared by IL12 and IL23 cytokines. The IL12 receptor is composed of two subunits, IL12RB1 (OMIM 601604) and IL12RB2 (OMIM 601642), which is homologous to the gp130 subunit^[77,78].

In a German population four *IL12B* SNPs (rs3212227, rs17860508, rs10045431 and rs6887695) were analyzed. The rs6887695 showed association with increased IBD susceptibility, and there was also trend for association with CD and UC. However just a trend was found for association of rs10045431 with UC. A haplotype of all four investigated SNPs showed a trend for association with CD^[79].

From these SNPs the rs6887695 was investigated in Spanish and Japanese population, but with different results. An association was found with CD (rs6887695) and UC susceptibility (rs6887695) in the Japanese cohort, but not to CD susceptibility in the Spanish cohort^[80,81]. Ex-

amining rs1363670 and rs6887695 SNPs in New Zealand population differential effect was found. Carrying the rs1363670 C variant increases risk for CD, while carrying the rs6887695 C variant decreases risk for CD^[82].

In a British cohort an association was found at rs6556416, which encodes a subunit shared by IL12 and IL23. Thus, the Th17 pathway seems as relevant to UC and CD^[83].

IL23

IL23 is a disulfide-linked heterodimer of the p40 (IL12B) and p19 (IL23A, OMIM 605580). IL23 interacts with a receptor composed of IL12RB1 and IL23R chain (IL23R, OMIM 607562)^[78]. IL23 functions in innate and adaptive immunity to regulate Th17 function and expansion^[84]. In addition, this cytokine induces CD8⁺ memory T cells to proliferate and produce IL17. Dysregulation of the IL23/IL17 immune axis has been linked to immunopathology and autoimmune inflammation, like IBD. *IL23R* polymorphisms play role in many autoimmune diseases^[85-87] especially in IBD^[88]. Polymorphisms in the *IL23R* represent one of the strongest associations in CD, and they have also been linked to the pathogenesis of UC^[89].

The *IL23R* gene was identified as a CD susceptibility gene in a North American population. Several independent functional SNPs of the gene and its neighboring region were determined, several were found susceptible to (rs10889677, rs11209032, rs11465804, rs11805303, rs1495965, rs2201841, rs1004819) and the others were protective (rs10489629, rs11209026, rs1343151, rs7517847) against IBD in non-Jewish subjects^[89]. After the primary publication, numerous replication studies have been published these *IL23R* genetic polymorphisms in IBD.

From the susceptibility variants the rs1004819 and rs1495965 were found as important risk factors for CD in Koreans^[90]. Similarly to these results the rs1004819 had the most significant association with CD in Germans, and the another rs10889677, rs2201841, rs11209032 showed increased genotype and allele frequencies comparing the CD cohort to the controls^[91]. Positive association was described of *IL23R* rs10889677 and rs1004819 SNPs with CD in Brazilian population, where the allele frequencies of the patients' group differ significantly from the controls^[92]. Another susceptibility factors were studied in Chinese cohort and the findings showed that rs7530511 and rs11805303 of *IL23R* gene showed positive association with UC susceptibility^[93]. In Jiangsu Han population the rs11805303 was found as a susceptibility polymorphism with UC too^[94].

The protective variants of the *IL23R* gene were analyzed in different populations. Association with rs11209026 and rs7517847 SNPs were confirmed in English subject, where the most significant SNP was the rs7517847^[95]. Similarly in Spanish population the rs7517847 and the rs11209026 showed association with IBD too, the rs7517847 showed the most protective effect against CD and UC^[80,96]. In another study the rs11209026 coding variant was found as a strong protection against CD in German pediatric CD patients^[97].

Five polymorphisms were analyzed in Hungarian IBD population (rs1884444, rs11805303, rs7517847, rs2201841, rs10889677 and rs11209032)^[98]. The rs2201841 and rs10889677 homozygous variants confer risk for the disease, while rs7517847 GG genotype has a protective effect against the development of CD. In Hungarian CD population two *IL23R* gene risk variants the rs2201841 and rs1004819 were found to be a susceptibility factor for CD^[99]. In another study with Hungarian CD patients increased prevalence of the homozygous rs10889677 AA and homozygous rs2201841 CC genotypes were found. The rs10889677 AA genotype was significantly increased in CD patients. The logistic regression analysis showed the AA genotype represents an independent risk factor for the development of CD^[100].

IL27

IL27 is a heterodimeric cytokine consisting of Epstein-Barr virus-induced gene 3 (EBI3, OMIM 605816) and p28 (also known as IL30, OMIM 608273). It binds a unique receptor subunit IL27RA (OMIM 605350), which is associated with gp130 (IL6ST, OMIM 600694), a common chain utilized by IL6 family cytokines. IL27 suppresses Th2 and Th17 differentiation and proliferation^[101,102].

In a Korean population the -A965G SNP was described as a susceptibility factor for IBD^[103]. In a GWAS five new regions were identified near the *IL27* gene associated with early-onset IBD susceptibility (rs8049439, rs2412973, rs1250550, rs4676410 and rs10500264)^[104].

IL6-LIKE CYTOKINES

Cytokines in this family (IL6, IL11, IL27 and IL31) signal through receptors containing gp130 which are commonly referred to as the IL6-like or gp130 utilizing cytokines family^[105]. They show pleiotropic biological activities with immune, hematopoietic, and neural systems^[105].

IL6

IL6 (OMIM 147620) is a multifunctional, pleiotropic cytokine involved in regulation of immune responses, acute-phase responses, hematopoiesis, and inflammation. IL6 signals through a cell-surface type I cytokine receptor complex consisting of the ligand-binding IL6R chain (OMIM 147880), and the shared signal-transducing component IL6ST (also called gp130; OMIM 600694)^[106].

English and Swedish children with CD and *IL6-174* GG genotype were more growth retarded at diagnosis and had higher levels of the IL6-induced inflammatory marker C-reactive protein (CRP) than children with GC or CC genotypes, concluded that *IL6-174* genotype mediates growth failure in CD^[107]. In an Irish cohort from Dublin, significant difference was found in the frequency of *IL6-174* genotypes in the UC group compared with the CD group^[57]. In a Caucasian population from Canada the same polymorphism was analyzed in CD and UC patients. There were significant difference IBD, UC and CD susceptibility, but it has influence on the clinical phe-

notype of CD^[108]. In a Spanish population^[109] homozygous for the *IL6 G-174C* polymorphism showed six-fold higher risk for CD. The GG genotype is associated with a greater production of IL6 compared with GC or CC genotypes^[110].

IL17 FAMILY

This cytokine family is a recently discovered group of cytokines with six members (IL17A, IL17B, IL17C, IL17D, IL17E and IL17F). IL17A was the original member of this family. The others were discovered primarily from the genome sequences within a short time-period (2000-2002), and were sequentially named in the order of discovery^[111-114]. They share the highest amino acid sequence homology and perform distinct biological functions^[115].

IL17

IL17A (OMIM 603149) acts on a variety of cells, which respond by upregulating expression of proinflammatory cytokines, chemokines, and metalloproteases. It is involved in the development of autoimmunity, inflammation, and tumors. IL17E (IL25, OMIM 605658) is an amplifier of Th2 immune responses. IL17F (OMIM 606496) is mainly involved in mucosal host defense mechanisms. The functions of IL17B (OMIM 604627), IL17C (OMIM 604628), and IL17D (OMIM 607587) are still largely elusive. Increased levels of IL17A^[116] and IL17F^[117] have been found in patients with IBD. It inhibits the proliferation of IECs, suggesting it might interfere with the repair mechanism important for the maintenance of the tissue integrity^[118].

The IL17 receptor (IL17R) family includes five members: IL17RA (OMIM 605461), IL17RB (OMIM 605458), IL17RC (OMIM 610925), IL17RD (OMIM 606807), and IL17RE (OMIM 614995)^[119].

In a Japanese UC patients the rs2275913 polymorphism in the *IL17A* gene and the rs763780 in the *IL17F* gene were analyzed. The frequencies of -197A/A and 7488T/T genotypes were found significantly higher in UC patients than in controls^[120]. In a Caucasian (German) population, despite the increased colonic IL17F expression in CD, any significant differences could be found in the frequency of rs763780 on IBD susceptibility^[117].

INTERLEUKINS WITH CHEMOKINE ACTIVITY

This group contains only two ILs, the IL8 and IL16^[13].

IL8

IL8 was identified first as a neutrophil-specific chemotactic factor and later classified as a member of the CXC chemokine family^[121]. Its receptors are: CXCR1 (IL8RA) and CXCR2 (IL8RB)^[122]. IL8 plays crucial role in the chemotaxis and migration of neutrophils, monocytes, lymphocytes, and fibroblasts^[123].

In a Polish population significant association was

found between the genotype frequencies for the heterozygote of the *IL8* T-251A and IBD. When IBD patients were subdivided in UC and CD subgroups this association was also observed. Significant difference was found between the A allele and the UC and CD cases, but not in the summarized IBD group^[124]. In a Chinese population any association was found between the T-251A polymorphism and UC^[125]. They also investigated the role of other polymorphisms of the *IL8* gene and its impact on the level of IL8 in serum. The frequency of the -353A/-251A/+678T haplotype was significantly higher in UC patients than in healthy controls. This haplotype tends to be more common in severe UC patients than in mild to moderate cases^[125].

IL16

IL16 (OMIM 603035) is a proinflammatory cytokine, which inhibits T-cell proliferation, promotes Th1-mediated responses, and reduces Th2-mediated inflammation^[126]. IL16 mediates its biological activity *via* CD4 molecule, which is present on T cells, monocytes, macrophages, and dendritic cells. Patients with CD have elevated levels of IL16^[127].

Regardless of the disease phenotype or the site of intestinal involvement, the T allele and the TT genotype in the *IL16* promoter region T-295C were found significantly increased in German CD cohort, but not in UC patients^[128].

TH2-LIKE CYTOKINES

Cytokines produced during the induction and function of Th2 response include IL4, IL5, IL9, IL13, IL25 (IL17E), IL31, and IL33^[13].

IL13

IL13's (OMIM 147683) receptors are IL13RA1 (OMIM 300119) and IL13RA2 (OMIM 300130), signaling occurs *via* the IL4R complex type II, which consists of IL4RA and IL13RA1. IL13RA2 acts as a decoy receptor of IL13. IL13 activates the same signal transduction pathways as IL4 and induces IgE production, influences eosinophils and cause their prolonged survival, activation, and migration to inflammatory lesions^[129]. IL13 plays an opposite role to IL8. In monocytes and macrophages, it inhibits the secretion of pro-inflammatory mediators such as prostaglandins, reactive oxygen species (ROS) and nitrogen species, TNF α , IL1, -6, -8, and -12^[130].

Presence of the *IL13* -1112 CT (rs1800925) genotypes in a Polish population showed higher risk of IBD as well as UC occurrence. The statistically significant differences in the T-allele distribution were observed in all the investigated groups^[124].

CONCLUSION

In this review we discuss IL and ILR gene polymorphisms which contribute to IBD, CD or UC in different ethnical

population. The cytokine network is highly complex with interactive cascades of gene activation and suppression. Not only the *IL* and *ILR* gene polymorphisms are in relation with IBD pathogenesis but also the downstream signaling components of several ILs (*i.e.*, JAKs, STATs) which could be potential targets of novel treatment strategies. Many IBD loci are also implicated in other immune-mediated disorders, most notably with ankylosing spondylitis and psoriasis^[9]. Since the individual associations may be non-informative, specific combinations of cytokine genotypes might predispose to disease susceptibility or outcome. Therefore, polymorphisms in cytokine genes and receptors should not in all cases be studied strictly in isolation. More complete understanding of the immunopathogenic role of the various ILs in intestinal inflammation will help in the development of more effective novel therapeutic strategies in IBD. Albeit genotyping these interleukin variants are often offer on the palette of several direct-to-costumer companies, their diagnostic or therapeutic clinical use is very limited due to the limited clinical utility and validity of them. Meanwhile, the next generation techniques in combination with the data analysis by systems-biology approach hopefully will contribute to the personalized therapy of the patients in the near future.

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Trefoil factors in inflammatory bowel disease

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also in protecting mucous epithelia from a variety of insults. This review describes the trefoil factor family and the role of the peptide family in relation to inflammatory bowel disease (IBD), and we summarize the current knowledge of their expression, possible function and potential pharmacological role in IBD.

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Abstract

Inflammatory bowel disease (IBD), which comprises ulcerative colitis and Crohn's disease, is characterized by inflammation of the gastrointestinal tract. The trefoil factors 1, 2, and 3 (TFF1-3) are a family of peptides that play important roles in the protection and repair of epithelial surfaces, including the gastrointestinal tract. TFFs may be involved in IBD pathogenesis and are a potential treatment option. In the present review, we describe the TFF family and their potential role in IBD by summarizing the current knowledge of their expression, possible function and pharmacological role in IBD.

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Key words: Trefoil factors; Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Inflammation

Core tip: Ulcerative colitis and Crohn's disease are characterized by mucosal inflammation. The trefoil factor (TFF) family consists of three peptides, TFF1, TFF2 and TFF3, and all are widely distributed in the mucous membranes of the gastrointestinal tract. The TFFs facilitate a significant role not only in mucosal repair but

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are the two most common inflammatory bowel diseases (IBDs). The etiologies of both diseases are unknown but are considered to be multifactorial, involving the genetic composition of an individual, the commensal gut flora and the environment^[1].

Studies of the mucosal barrier indicate that trefoil factors (TFF) facilitate a significant role not only in mucosal repair but also in protecting mucous epithelia from a variety of insults in the gastrointestinal tract^[2]. In this respect, repair is essential for preventing inflammation and ulceration. In conjunction with other mechanisms, several products that are primarily secreted from the goblet cells, including TFF and mucins form the innate immune response and first line of defense in the mucus layer. How this fully occurs is still only partly understood^[3].

In mammals, the trefoil factor family consists of three peptides: TFF1, TFF2 and TFF3; all three are widely distributed in the gastrointestinal tract and are present in virtually all mucous membranes. The importance of TFFs in the protection and repair of epithelial surfaces is well established^[4].

TFF1 and TFF3 each have one trefoil domain, while

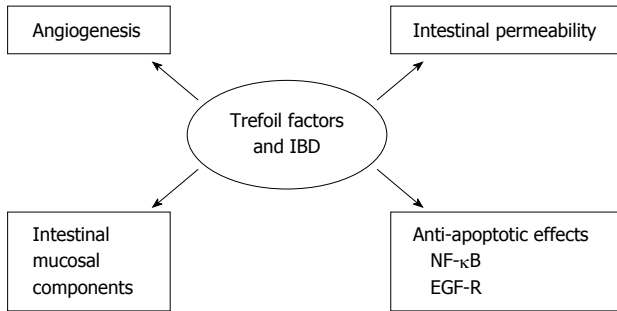


Figure 1 Role of trefoil factors in inflammatory bowel disease. The potential mechanisms involving anti-apoptotic properties, migration and invasion, angiogenesis, and interaction with mucins. IBD: Inflammatory bowel disease; EGF-R: Epidermal growth factor receptor; NF-κB: Nuclear factor-kappa B.

TFF2 has two trefoil domains. The trefoil domain is characterized by a sequence of amino acid residues, in which 6 cysteines are linked by 3 disulphide bonds to form the “trefoil” disulphide loop structure or the clover-like shaped structure^[5]. The resistance of the peptides to proteolytic digestion, acids and thermal degradation seems to be caused by the compact trefoil structure of the peptides^[6,7]. TFF1 and TFF3 only contain one trefoil domain but have a seventh free cysteine, which is essential for the formation of dimers^[6]. It is not clear whether the main part of naturally occurring TFF1 and TFF3 consists of monomers or dimers^[2].

TFF2, formerly known as the Pancreatic Spasmolytic Polypeptide, was the first TFF to be isolated in the early 1980s from a side-fraction obtained during the purification of insulin from porcine pancreas^[7]. The human homologue of TFF2 is produced primarily by mucus neck cells in the body and in antral glands of the stomach, while a small amount is expressed in Brunner’s gland in the duodenum^[8,9]. The cloning of an estrogen-regulated gene from the MCF-7 human breast cancer cell line resulted in the identification of pS2, which is today known as TFF1^[10]. TFF1 is also produced in the stomach by superficial gastric foveolar cells^[11]. It was also discovered that these peptides share a new sequence motif, named the trefoil domain^[5]. A third trefoil factor, TFF3, was identified in 1991 as a rat cDNA sequence^[12] and a human cDNA sequence in 1993^[13] that was initially known as the intestinal trefoil factor (ITF). TFF3 is expressed in the goblet cells of the small and large intestine^[14] and is co-produced and secreted with mucin (MUC)2^[15].

This review describes the TFF family and the role of this family as it relates to IBD and summarizes the current knowledge of their expression, possible function and pharmacological role in IBD.

FUNCTIONAL CHARACTERISTICS OF TFFS

The physiologically relevant functions of TFFs are not clear, and the important question of how TFFs work remains unresolved. Do they work by cross-linking

with mucins, *via* a receptor, or in a completely different way? Data suggest that TFFs may have multiple cellular functions^[16,17]. Below, we describe the potential mechanisms involving anti-apoptotic properties, migration and invasion, angiogenesis, and interaction with mucins (Figure 1).

Anti-apoptotic properties are very important for epithelial restitution, where epithelial cells must migrate over the denuded area of the gut mucosa. In this process, the epithelial cells are vulnerable to apoptosis or anoikis, which is the form of apoptosis that is induced by anchorage-dependent cells detaching from the surrounding extracellular matrix. TFFs have been found to have anti-apoptotic effects in several cell lines^[18,19], and this effect has been supported by the finding that TFF3-deficient mice have increased numbers of apoptotic cells in their colonic crypts^[17]. Furthermore, TFF3 has anti-anoikis effects on intestinal epithelial cells *via* its activation of nuclear factor-kappa B (NF-κB)^[20]. This effect of TFF was dependent on the activation of epidermal growth factor receptor (EGFR) and required TFF3 dimer^[18].

An abnormal distribution or expression of tight junction proteins in gastrointestinal epithelial cells, which causes barrier dysfunction, is thought to be involved in IBD pathogenesis^[21]. The effect of TFF3 on increased intestinal permeability and its association with tight junction proteins was evaluated in an *in vitro* intestinal epithelia barrier model in which colorectal epithelial cells were treated with platelet-activating factor (PAF). The analysis revealed that TFF3 suppressed the PAF-induced down-regulation of the tight junction proteins claudin-1 and zonula occludens-1. These proteins maintain the tight junction’s integrity and intestinal barrier function, and TFF3 thereby decreases mucosal permeability^[22]. TFF3 induces the recovery of tight junction protein changes, which contributes to the TFF3-mediated stabilization and maintenance of intestinal epithelial barrier function. The findings may provide new insight into the protective functions of TFF3 in epithelial cells and demonstrate its potential for treatment of IBD^[22,23].

The proliferative phase of wound healing is characterized by angiogenesis and pro-angiogenic properties, which are dependent on cyclooxygenase-2 and EGFR signalling and have been described for all TFFs^[24].

Alterations in the intestinal mucous components may impair the barrier function of the mucin layer and may be a contributing factor to IBD^[25]. In IBD, the mucin types and expression are affected by several factors. For example, the numbers of mucin-producing goblet cells are reduced in active disease and changes in the thickness and composition of the mucous gel layer may occur^[26]. Although MUC2 is the major colonic mucin, alterations in the composition and concentrations of colonic mucins occur in IBD^[26,27].

Several studies support the hypothesis that TFFs interact with mucins to enhance the mucosal barrier. TFFs and mucins are co-localized in the gut. When TFF3 and mucin were combined, they were more effective in pro-

tecting epithelial cells in an *in vitro* model of epithelial barrier, which indicates a joint effect in mucosal protection^[28]. The TFFs may act differently when coupled with specific mucins, which is supported by the finding that each TFF co-localizes with its own unique mucin type in the ulcer-associated cell lineage (UACL) and normal gastrointestinal mucosa. For example TFF1 couples with MUC5AC, TFF2 couples with MUC6 and TFF3 couples with MUC2^[29].

The combination of mucins and TFFs has been demonstrated to protect cell monolayers against injurious agents by increasing mucus viscosity and decreasing proton permeation^[28,30]. TFF2 in particular has been shown to increase the viscosity and elasticity of porcine gastric mucus and may contribute to a more resilient protective barrier than TFF3. Conversely, TFF1 and TFF3 do not increase the viscosity of mucus but instead form small complexes with the mucins^[31], which may be beneficial in the intestines. TFF1 binds to the von Willebrand Factor C domain of MUC2^[32]. TFF3 was recently reported to form a disulfide-linked heteromer with IgG Fc binding protein, which could contribute to the stability of the mucin network in the mucus layer by interacting with MUC2 mucin^[33].

In addition to the direct stabilization of the surface mucous layer, TFF-mucin interactions have also been shown to promote cellular effects such as cell migration and NO production^[34,35].

TFFS IN EXPERIMENTAL IBD

In animal models of IBD induced by the intrarectal administration of 5% acetic acid, various and conflicting patterns of altered/increased TFF expression have been observed^[36]. A possible explanation for the conflicting results observed in studies exploring the effects of TFFs in the treatment of gastrointestinal damage may be that different TFF forms, dosages and administration routes have been used in colitis models.

In general, the expression pattern of the TFFs in animal models differs from the pattern observed in humans, but the models have been very useful for investigating, for example, the temporospatial expression of TFFs after the induction of damage and the possible use of TFFs for pharmacologic intervention in IBD.

In vivo studies have clearly shown that TFFs have protective and healing effects when given exogenously following either enteral or parenteral administration. This finding suggests that TFFs might be useful in the IBD treatment. In this instance, rodent colitis models have been useful for examining the relationship between intestinal damage and the expression of the TFFs and thus examining the role of exogenously added TFFs in epithelial repair during instances of injured mucosa.

Mashimo *et al.*^[37] showed that mice lacking TFF3 had impaired mucosal healing, with poor epithelial regeneration after injury; those mice died from extensive colitis after oral administration of dextran sulfate sodium (DSS),

an agent that causes mild epithelial injury in wild-type mice. The same was observed following chemotherapy and radiation-induced damage^[38]. In addition, luminal treatment with recombinant TFF3 (rTFF3) restored the capacity for restitution in TFF3-knockout mice exposed to DSS and radiation-induced damage^[37,38].

Although several animal studies have documented the effect of treatment with TFFs, the optimal administration route for treatment with TFFs remains unclear. In animal models of gastric ulceration, both oral and systemic treatments with TFFs are effective for protection, prevention and healing^[39-43]. Subcutaneous infusions of recombinant TFF2 (rTFF2) or porcine TFF2 (pTFF2) decreased acute gastric ulceration damage by 50% without changing the gastric acid secretion^[39,40]. In the gastric ulceration model, both orally and subcutaneously administered TFF2 had an effect; however, both treatments aggravated duodenal ulcerations. After oral administration, pTFF2 is bound to the mucus layer of the stomach and small intestine, but it does not reach the colonic mucosa^[41]. The same dose of pTFF2, given subcutaneously, was superior to oral pTFF2 treatment. When administered orally as a prophylactic treatment, hTFF2 had a protective function in Non-steroidal anti-inflammatory drug (NSAID)-induced damage in rat gastric mucosa^[42], whereas both rTFF2 and rTFF3 prevented ethanol- and indomethacin-induced gastric injury when given up to 2 h before injury. However, following intraperitoneal administration, the rTFF2 treatment had no effect^[43].

The effects of TFFs have also been demonstrated in animal models of intestinal inflammation and damage. Here, the optimal route of administration is unclear, with some studies favoring oral treatment and others favoring systemic treatment^[44-46]. In a DSS-induced experimental colitis model, pre-treatment with subcutaneously or intracolonic administered TFF2, ameliorated the clinical course of this chemically induced colitis, with the luminal route being superior to the parenteral route^[44]. In another study, the distribution profile of subcutaneously and intraperitoneally administered TFF3 was very similar to the intravenous distribution, with a high uptake of tracer in the kidney and gastrointestinal organs. TFF3 availability was slightly faster following *sc* administration than following *ip* administration, and both administration routes would yield comparable pharmacological effects^[45]. The molecular forms of TFFs also seem to play a role. In a DSS colitis model and in a model of colitis induced by the intraperitoneal injection of mitomycin luminal treatment with dimeric TFF3 was effective, whereas treatment with monomeric TFF3 had no effect. It is worth mentioning that a systemic TFF3 monomer treatment intensified the mucosal insults^[47]. In a previous study, the subcutaneous administration of the hTFF1 dimer was proven to be more compelling than the TFF1 monomer^[48].

The DSS model is considered to be suitable for studying acute epithelial damage, but the model lacks the chronic inflammation characteristics of IBD. In colitis models that are more representative of IBD, such as the

dinitrobenzene sulphonic acid model, hTFF2 has shown enhancing effects on colonic epithelial repair and a decrease in local inflammation after luminal application. In addition, endogenous concentrations of TFF2 and TFF3 were increased in the active phase of colitis and reduced to basal levels after hTFF2 treatment^[49].

The chronic production of NO *via* inducible nitric oxide synthase (iNOS) leads to tissue damage and inflammation. In a study involving local intracolonic hTFF2 treatment, the *in vitro* inhibition of NO and iNOS in monocytes was observed, along with a reduction in the levels of the damaging reactive oxygen species and a decrease in colitis. These findings further indicate that TFFs exert a positive effect on mucosal protection^[50].

In combination therapy, TFF3 and EGF act in a synergistic manner to stimulate cell migration *in vitro* and can potentially provide a more effective and safe approach for treating intestinal ulcerations^[51]. This may reduce the degree of colonic injury and may prove to be useful when treating colitis in patients with a disease that is beyond the reach of enema therapy^[46].

Poulsen *et al.*^[52] showed that orally given TFF did not reach the colon. Systematically administered TFF2 and TFF3 bind to the gastric mucosal surface and are transported to the lumen. Whether this occurs in the colon is uncertain, but it seems that less of the systemically administered TFF is present in the colon than in the stomach^[52,53]. The intragastric administration of the TFF1-secreting *Lactococcus lactis* (*L. lactis*) in DSS-induced colitis was followed by the active delivery of TFF at the colonic mucosa. A significant protective effect was observed, which may represent a new therapeutic approach that involves the *in situ* secretion of TFF by orally administered *L. lactis*^[54].

In another recent attempt to improve the application of TFFs, a recombinant adenoviral vector containing the human ITF (*hITF*) gene was constructed and shown both to promote cellular migration in an *in vitro* intestinal wound model and to improve the healing of intestinal mucosal injury^[55].

REGULATION OF TFF EXPRESSION IN IBD

Several studies have shown that the cytokines and transcription factors that are related to the immune system and important in IBD can regulate the expression of TFFs and *vice versa*.

The tumor necrosis factor alpha (TNF- α) triggering of NF- κ B activation is known to be a proinflammatory factor in the pathogenesis of IBD and may contribute to the development of ulcerations. Toll-like receptor-4 (TLR4)/NF- κ B expression is essential for the activation of human intestinal epithelial cells and the subsequent expression of cytokines. Using cell culture studies, it was shown that both TNF- α and NF- κ B induced the down-regulation of TFF3 by repressing transcription and in experimental colitis, the increase in the epithelial expression of NF- κ B coincided with reduced TFF3 expression

during the acute phase^[56].

In a more recent study, the intraperitoneal application of rTFF3 promoted a protective effect against colitis (trinitrobenzene sulphonic acid-induced) and was accompanied by a reduction in TNF- α expression in the colonic endothelium. The protective effect was also paralleled by a reduction in TLR4/NF- κ B expression, indicating that hTFF3 may have therapeutic potential through the inhibition of the TLR4/NF- κ B signaling pathways^[57].

Podolsky *et al.*^[58] investigated the possible linkage between TLR2 that plays a key role in the innate immune system as well as in the digestive system. This possible linkage was studied for TFF3 in a DSS-induced colitis model and for TLR2 and TFF3 in knockout mice models. The oral administration of a TLR2 agonist in TFF3 and TLR2 knockout mice causes anti-apoptotic protection of the TFF3 stress-induces inflammatory intestinal mucosa. Recombinant TFF3 administration decreased morbidity and mortality during acute colonic injury in a TLR2-deficient mice model. These findings imply that TLR2 exerts diverse mucosa-protective properties in different epithelial cell types, critically suppressing mucosal apoptosis and the associated leukocyte influx during acute colitis by regulating TFF3 in goblet cells.

Several studies have indicated that TFF expression may be regulated *via* the EGFR; TFFs and EGF are co-expressed in the UACL cell line^[59]. The transcription of TFF1 is enhanced by EGF^[60], and EGFR activation is required for the auto- and cross-induction of TFFs and for the anti-apoptotic effect of TFF3^[18]. Additionally all TFFs have been shown to cause transient phosphorylation of the EGFR^[61]. However, no binding to the EGFR has been demonstrated, and the mechanisms remain unclear.

In a recent study, it was shown that dietary supplementation with conjugated linoleic acid, which may have anti-inflammatory effects, protected against DSS-induced colitis in a process involving the induction of TFF3^[62].

Overall, multiple studies have investigated, with conflicting results, the relation between TFFs and cytokines as well as the transcription factors related to the immune system. More studies are clearly needed to describe the regulation of TFFs in IBD and thus pave the way for drug development.

TFFS IN CLINICAL STUDIES

Although multiple *in vitro* and animal studies since the discovery of TFF 30 years ago have documented the crucial role of TFFs in the epithelial restitution of the gut, few studies have been performed in IBD patients to investigate the clinical potential of TFF in IBD.

TFF are expressed in several tissues that contain mucus-secreting cells, but they are most markedly expressed in the gastrointestinal tract. At this site, each TFF is co-localized with its unique mucin type. For example, TFF1 is co-localized with MUC5AC, TFF2 is co-localized with MUC6, and TFF3 is co-localized with MUC2^[63,64]. TFF1 and TFF2 are primarily located in the stomach^[8,11], where-

as TFF3 is predominantly present in the mucous cells of the small and large intestine^[14]. Several studies have documented the supportive and protective functions of TFFs in the human gastrointestinal tract. Those studies have shown the up-regulated expression of all three TFFs at the site of mucosal damage in IBD^[65,66], peptic ulcer^[67] and the neoexpression of TFF1 in UC with histologically severe disease^[68].

The UACL, which occurs at sites of chronic gastrointestinal ulceration including IBD expresses a number of peptides that have been implicated in the repair of damaged mucosa, such as the TFFs^[13,69]. In small intestinal Crohn's disease, TFF2 mRNA is expressed in the acinar and proximal duct cells, while TFF1 mRNA and peptide are found in the distal duct cells and in the surface cells^[65]. As in normal gastrointestinal mucosa, the co-localization of specific TFFs and mucins is observed in IBD^[29,68].

The co-localization of TFF3 with DMBT1 in IBD, which has been proposed to have a role in cell differentiation and growth, indicates that DMBT1-TFF3 interactions may play a role in IBD^[70].

Quantitative measurements of TFFs have been important tools for elucidating the biological functions of the peptides and exploring their role as biomarkers for IBD. Larger than normal serum concentrations of TFF2 and TFF3, *i.e.*, 2000 to 10000 and 140 to 500 times higher, respectively, have been measured in bowel discharges^[71]. All three TFFs are present in sera from healthy individuals^[72]; in line with immunohistochemical studies showing increased expression in IBD, the sera concentrations of the peptides were also elevated in IBD patients^[73-75]. The TFF3 concentrations were significantly higher in UC patients and their levels correlated with the clinical and biochemical parameters of disease activity.

Because of large biological variations, measurements of TFFs are not useful as clinical biomarkers for disease activity in CD and UC^[72,75]. However, quantitative measurements may still be important and should be included in continued research in the area.

In clinical studies TFF peptides are considered promising for the treatment of inflammatory conditions of mucous membranes. In IBD the effect of TFF3 enemas, given in combination with oral mesalazine in patients with mild-to-moderate left-sided UC, have been tested. UC patients were given a total daily dose of 750 mg of dimeric rTFF3 in 75 mL enemas (dosage concentration of 10 mg/mL), similar to the luminally administered dose used in animal models of gut injury that has proven effective. The TFF3 enema was well tolerated, but in this first human study no additional benefit of TFF3 treatment was detected compared to the effect of 5-aminosalicylic acid treatment alone^[76]. One possible explanation may be the rapid decay of TFFs observed in the colon^[71]. In future trials, the systemic route should be explored.

CONCLUSION

Since the discovery of the TFFs, a number of animal studies and studies on UC and CD patients have shown

that the peptides are linked to inflammatory conditions in the gut. Although a significant role in mucosal protection and repair has been established for the TFFs, the full knowledge of their biological functions in IBD remains elusive. The quantitative measurements of the peptides in patients with IBD have been less promising, due to large inter-individual variations, and their future use as biomarkers in IBD is uncertain. Future studies are needed to show whether the peptides have a potential as a novel therapeutic in IBD. Additionally further identification of the regulatory mechanisms that can affect TFF expression may aid in the development of new drugs for treating IBD.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Biomarkers in inflammatory bowel diseases: Current status and proteomics identification strategies

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Abstract

Unambiguous diagnosis of the two main forms of inflammatory bowel diseases (IBD): Ulcerative colitis (UC) and Crohn's disease (CD), represents a challenge in the early stages of the diseases. The diagnosis may be established several years after the debut of symptoms. Hence, protein biomarkers for early and accurate diagnostic could help clinicians improve treatment of the individual patients. Moreover, the biomarkers could aid physicians to predict disease courses and in this way, identify patients in need of intensive treatment. Patients with low risk of disease flares may avoid treatment with medications with the concomitant risk of adverse events. In addition, identification of disease and course specific biomarker profiles can be used to identify biological pathways involved in the disease development and treatment. Knowledge of disease mechanisms in general can lead to improved future development of preventive and treatment strategies. Thus, the clinical use of a panel of biomarkers

represents a diagnostic and prognostic tool of potentially great value. The technological development in recent years within proteomic research (determination and quantification of the complete protein content) has made the discovery of novel biomarkers feasible. Several IBD-associated protein biomarkers are known, but none have been successfully implemented in daily use to distinguish CD and UC patients. The intestinal tissue remains an obvious place to search for novel biomarkers, which blood, urine or stool later can be screened for. When considering the protein complexity encountered in intestinal biopsy-samples and the recent development within the field of mass spectrometry driven quantitative proteomics, a more thorough and accurate biomarker discovery endeavor could today be performed than ever before. In this review, we report the current status of the proteomics IBD biomarkers and discuss various emerging proteomic strategies for identifying and characterizing novel biomarkers, as well as suggesting future targets for analysis.

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Key words: Inflammatory bowel disease; Biomarker; Proteomics; Citrullination; Ulcerative colitis; Crohn's disease; Posttranslational modification

Core tip: Establishing the correct diagnose of Crohn's disease and ulcerative colitis (UC) patients remains troublesome, and correct and early medication is critical. No reliable biomarkers have been implemented in clinical usage, to distinguish between Crohn's disease patients and UC patients. Considering the protein complexity encountered in intestinal biopsy samples and the recent development within the field of quantitative proteomics, submitting the intestinal mucosa to a more thorough analysis has the potential to reveal new biomarkers.

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INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic gastrointestinal disorders. The two most common forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Both disorders have great impact on the life quality of the affected individuals and for society, measured on lost labor and expenses to the health care system. Furthermore, new epidemiological data published in 2013 found that the incidence and prevalence of the diseases are still increasing^[1]. The etiologies of CD and UC remain unclear, but involve a complex interplay between genetic and environmental factors^[2-7]. The diagnosis can be delayed several years and may be difficult to make even for trained physicians, as no biomarkers or commercial tests capable of discriminating CD from UC patients have been implemented in clinical use^[8-10]. Furthermore, an early and accurate diagnosis of IBD-patients is crucial, as *e.g.*, CD patients with extensive and deep ulcerations have a 5-fold higher risk of requiring colectomy compared to CD patients without extensive and deep ulcerations^[11]. From 357 CD patients analyzed with computed tomography enterography, penetrating disease was found in 21% of the patients and extraintestinal manifestations in 19%^[12,13]. Hence, there is a need for reliable and usable biomarkers for the early and better diagnosis and prognosis of the IBD diseases^[4,8,14-18].

GENOMIC, TRANSCRIPTOMIC AND PROTEOMIC BIOMARKERS

In 2001, an NIH group defined a biomarker as "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention."^[19], usable for diagnostics, monitoring disease prognosis and disease monitoring and prediction. The human genome contains the code for the expressed gene products, including the proteins. Proteins function as the building blocks of the human cells and tissue, and are responsible for the majority of the biological functions^[20]. Proteins, therefore, represent an obvious target for biomarker discovery studies. The human genome comprises approximately 20000 protein coding genes^[21]. During protein synthesis, the DNA code is first transcribed into different RNA transcripts. Each gene can give rise to several RNA transcripts resulting in a total of roughly 100000 different RNA transcripts^[22-24] (Figure 1), which in turn are translated into 100000 different proteins. After

translation, most proteins are covalently modified at least once^[25], and the final mature protein products are termed proteoforms. These so-called posttranslational modifications (PTMs) are often crucial to the correct physiological function of the given protein, and can determine activity state, localization, turnover and interaction with other proteins and substrates^[23,25,26]. More than 200 distinct biologically relevant PTMs have been identified^[27], so each RNA transcript can be more than 200 different proteoforms. The PTMs increases the complexity and diversity of the proteins tremendously (Figure 1). As a result, it is estimated that the human body contains more than one million different proteoforms^[23], which constitutes the human proteome (all expressed proteins).

When searching for biomarkers, it is possible to analyze the target sample on the DNA level, the RNA transcript level or the protein level. Techniques for studying an organisms DNA code (genome) or RNA transcripts (transcriptome) have the advantage that entire genomes and transcriptomes can be sequenced and studied with great sensitivity, precision and coverage, and a number of biomarkers have been found for various diseases. Using genomic sequencing techniques, several CD and UC loci have been known for more than a decade, and the studies have greatly increased our knowledge of the IBDs^[22,28,29]. Several cellular IBD-pathways have been identified, including pathways involved in barrier function, epithelial restitution, microbial defense, immune regulation, reactive oxygen species generation, autophagy, and finally various stress and metabolic pathways associated with cellular homeostasis, reviewed by Khor *et al*^[3]. However, as mentioned no IBD biomarkers capable of differentiating CD from UC have been implemented in daily clinical usage, and the impact of the genomic studies on the treatment and diagnosis of the IBDs has been questioned^[30-32].

Proteins represent an obvious target for biomarker discovery studies, and as PTMs dramatically increases the diversity and in many cases function of the mature proteins, they represent a promising area for IBD biomarker studies. PTMs are introduced after translation of the RNA transcripts (Figure 1), hence analyzing DNA and RNA transcripts does not directly provide information about the PTMs. A key technique capable of measuring absolute and relative protein quantification in complex protein mixtures in a high-throughput manner, as well as identify several PTMs, is bottom-up mass spectrometry (MS) based proteomics^[24,33]. Proteomics is the large-scale identification of proteins, and can often cover the study of all expressed proteins by an organism (the proteome). The bottom-up MS strategy is based on measuring the mass-to-charge ratios (m/z) of peptides derived from proteins which have been enzymatically cleaved into minor peptides. From the measured m/z 's the molecular weight of the intact peptides can be calculated^[25]. In addition to calculating the intact masses, the peptides are collided with an inert gas which fragments the peptides, and the fragment m/z 's are measured. The proteins in the sample are subsequently identified by searching the

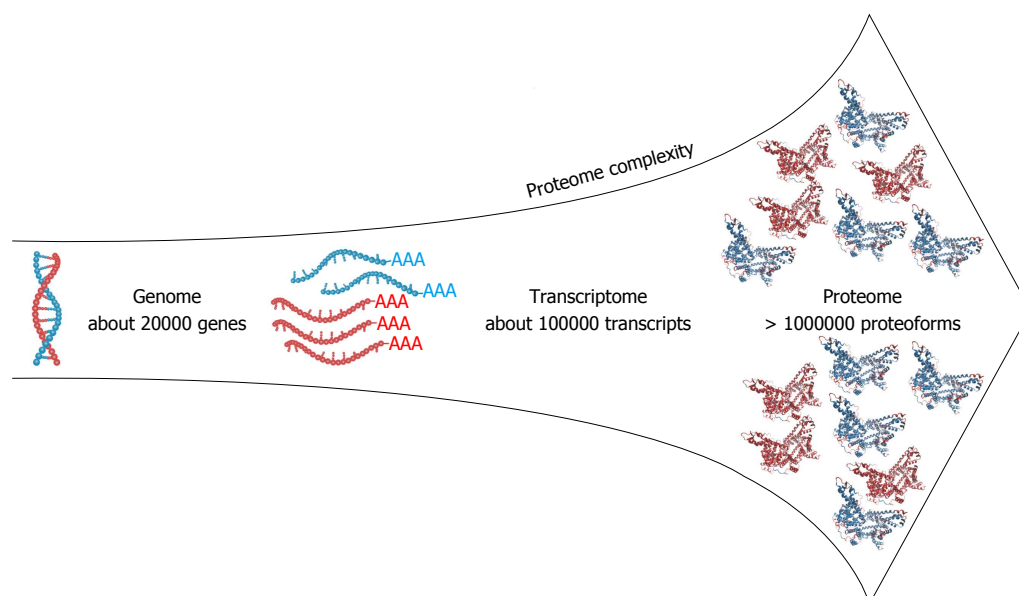


Figure 1 Major increase is encountered in the proteome complexity, from genes to RNA transcripts and finally to the mature, often posttranslational modification modified, proteins (proteofoms).

peptide masses and fragment m/z 's against an *in silico* generated database, inferred from a reference database of protein sequences. By matching the *in silico* calculated peptide masses and fragment m/z 's to the measured, the peptides and hence the proteins, are identified. For a more thorough description, we refer to the review by Steen *et al.*^[34]. The process can be performed in a quantitative manner to allow for relative or absolute quantitation of the proteins, using different strategies^[34]. MS can in this way be used to identify proteins, as well as PTMs that change the molecular weight of the protein and can provide the amino acid position of the modification^[25]. Previously, proteomics has been limited mainly by the speed and sensitivity of the mass spectrometers. However, recent development within the field of MS has allowed for the identification of nearly all expressed proteins of complex organisms, such as yeast, within a few hours of measuring time, identifying and quantifying several thousand proteins^[33,35]. When considering the protein complexity encountered in the human intestinal tissue, an obvious place to search for biomarkers, and the recent development in the field of MS, a thorough analysis of PTMs and protein abundances in healthy and diseased state could be conducted. Biomarkers found in the intestine could then be searched for in more easily obtained sample material, such as blood or stool^[6,10,31,36-39]. Antibodies to identified biomarkers for CD and UC found by proteomics can be generated for development of immunoassays and immunohistochemistry for evaluating the markers clinical use in routine tests less expensive than sequencing genomes, transcriptomes or MS driven proteomics.

This review reports known biomarkers for the IBDs, but will focus on the newly identified proteomics biomarkers and emerging proteomics strategies for identifying and characterizing novel IBD biomarkers.

DIAGNOSIS OF INFLAMMATORY BOWEL DISEASE AND KNOWN BIOMARKERS

Numerous biomarkers are known and used for the IBDs (Table 1); however, no single biomarker is able to diagnose IBD or to distinguish CD from UC patients with a high specificity and sensitivity^[8-10,14]. CD is characterized by chronic inflammation in any part of the gastrointestinal tract. Most commonly the terminal ileum or the perianal region are inflamed, and in a non-continuous manner. Histologically, CD shows thickened submucosa, transmural inflammation, fissuring ulceration and non-casating granulomas. UC, on the other hand, is characterized by inflammation limited to the colon, spreading continuously from the rectum and various distance proximal, and histology shows superficial inflammatory changes limited to the mucosa and submucosa with inflammation of crypts (cryptitis) and crypt abscesses^[3]. There is currently no single "gold standard" diagnostic test or examination to differentiate CD and UC. Instead, diagnosis is based on a combination of symptoms, clinical examinations, laboratory findings, radiology, and endoscopy with histology, which also is used to assess severity and to predict the outcome of disease. Even when the tests are performed by expert clinicians they can result in diagnostic uncertainty^[10,14,15,17,40]. This section will report some of the biomarkers commonly used to diagnose IBD. For a review of additional IBD biomarkers we refer to the work of Iskandar *et al.*^[41].

Antibodies and serum biomarkers

The two best-studied serological markers in IBD patients are anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-neutrophil cytoplasmic antibody (ANCA)^[41].

ASCA is an antibody with affinity for antigens in the

Table 1 Known common inflammatory bowel disease biomarkers

Biomarker	Specificity	Usability
Serum biomarkers		
ASCA	39%-79% of CD patients positive, 5%-15% UC patients ^[41-43]	14%-18% of controls tested positive, limiting the diagnostic value ^[44]
pANCA	20%-85% of UC patients positive, 2%-28% of the CD patients ^[41,42,45]	32% of controls tested positive, limiting the diagnostic value ^[44]
CRP	Marker for acute inflammation	Cannot differentiate CD from UC. However, usable for monitoring disease state ^[48-50]
Fecal biomarkers		
Calprotectin	Sensitive marker for intestinal inflammation ^[8,17,40]	Cannot differentiate CD from UC. Used to monitor disease state ^[17]
Lactoferrin	Can distinguish active IBD from inactive IBD and irritable bowel syndrome ^[60]	Unspecific for CD and UC. However, usable for monitoring disease state ^[60]

ASCA: Anti-*Saccharomyces cerevisiae* antibodies; ANCA: Anti-neutrophil cytoplasmic antibody; IBD: Inflammatory bowel diseases; UC: Ulcerative colitis; CD: Crohn's disease; CRP: C-reactive protein.

cell wall of the yeast *Saccharomyces cerevisiae*. In comparison to UC patients, CD patients are often positive for ASCA (Table 1)^[41-43]. However, a substantial amount of healthy controls are also positive for ASCA positive^[44], indicating that specificity and sensitivity for CD patients are relatively low; limiting the diagnostic value of the marker in differentiating CD from UC.

ANCAs are antibodies with affinity for neutrophil granules. The antibodies have been found in a variety of immune conditions, including Wegener's granulomatosis and rheumatoid arthritis (RA)^[4]. When staining for ANCA, different patterns have been observed for UC and CD patients using immunofluorescence microscopy (Table 1), and mainly UC patients display perinuclear ANCA (pANCA) staining compared to CD patients^[41,42,45]. Nonetheless, like the case of ASCA, a substantial amount of healthy controls are pANCA positive^[44].

Lastly, C-reactive protein (CRP) is one of several proteins that increase in serum upon acute phase IBD. CRP is almost exclusively produced in the liver, upon stimulation by interleukin (IL)-6, tumor necrosis factor (TNF)-alpha and IL-1-beta produced at the site of inflammation. As such, an increased CRP-level is a marker for inflammation, but is not specific for CD or UC^[8,40,46,47]. In some cases, but far from always, CD is associated with a strong CRP serum increase, whereas UC usually only results in a modest response. However, the difference insufficient to differentiate CD patients from UC patients^[48-50], and the reason for the different responses remains to be thoroughly accounted for^[40].

Other serum biomarkers used include white blood cell count, platelets, and albumin, which are all non-specific for IBD and can be seen in inflammatory diseases and cell stress^[40]. More CD serologic markers are described in

the review by Tamboli *et al.*^[51].

Fecal biomarkers

Stools are in direct contact with the inflamed intestinal area and site for the gut microbiome, both from which potential biomarkers are likely to originate. This is in contrast to serum biomarkers, which could increase on account of a variety of conditions, making stools an obvious place to search for biomarkers^[40]. Fecal markers are especially useful for the diagnosis of CD patients, where the inflammation is patchy, may affect any part of the gastrointestinal tract, and therefore might be missed by colonoscopy^[52]. The host-microbe interactions have been recognized as central for understanding human physiological diversity, and the human microbiome project has been launched to unravel the medical significance of the human microbiome^[53]. Several studies have identified certain bacterial groups which are more abundant (*Enterobacteriaceae*, *Ruminococcus gnavus*, and *Desulfovibrio*) or less abundant (*Faecalibacterium prausnitzii*, *Lachnospiraceae*, and *Akkermansia*) in IBD^[16], implicating that the host-microbe interaction might be involved, reviewed by Rosenstiel^[54]. Novel biomarkers with high sensitivity and specificity may, therefore, be identified from stools.

The two most commonly used fecal markers for IBD screening are calprotectin and lactoferrin (Table 1)^[8]. Calprotectin is a calcium- and zinc-binding protein occurring in large amounts in neutrophil granulocytes, where it accounts for 5% of the proteins. It is a very stable marker and is resistant to colonic bacterial degradation, and can be stored at room temperature for more than a week^[55]. The concentration of fecal calprotectin is proportional to the neutrophil cell infiltrate in the bowel mucosa, and it is a very sensitive marker for intestinal inflammation^[8,17,40]. However, calprotectin is not a specific marker for CD or UC, and increased levels can also be found with neoplasia, other forms of IBD, infections, and polyps^[17], as well as with use of non-steroidal anti-inflammatory drugs, increasing age^[56] and upper gastrointestinal disease, such as small bowel bacterial overgrowth^[57].

Lactoferrin is an iron-binding glycoprotein expressed by activated neutrophils^[58]. During inflammation, lactoferrin is released by the injured tissue and has been found to modulate inflammation and act in the defense against infections as a part of the innate immune system^[59]. It is resistant to degradation and proteolysis, and unaffected by freeze thaw cycles, making it a useful biomarker^[17]. As such, it is an ideal marker for intestinal inflammation. However, like calprotein it is unspecific for CD and UC, but can distinguish active IBD from inactive IBD and irritable bowel syndrome^[60]. Several studies report similar performance of calprotectin and lactoferrin tests^[6,60-64], and neither can be used to differentiate CD from UC with a high sensitivity and specificity.

To sum up, no reliable biomarkers exist usable as a single "gold standard". Therefore, to establish a diagnosis, histological examination of biopsies from the terminal ileum and colon is typically used in combination with

patient disease history and one or more of the above mentioned markers^[17,65]. Hence, much effort is invested in analyzing the IBDs using various strategies, to identify usable biomarkers and explain the disease etiologies.

KNOWN PROTEOMICS BIOMARKERS FOR INFLAMMATORY BOWEL DISEASE

Proteomics studies can be performed in a discovery-based manner, where relative protein abundance levels between two or more samples are detected, and PTMs can be identified. Recent development of proteomics platforms has brought the technology to the point where several thousand proteins can be identified and (relatively) quantified in a single analysis or a subset by targeted approaches^[6,10,31,36-39]. As inflammation takes place in the intestine, the gut-tissue represents an obvious place to look for novel biomarkers, which afterwards may be searched for in for example feces and blood and used as a disease marker. Several proteomic studies have successfully been aimed at identifying IBD biomarkers to investigate disease etiologies and aid in establishing the correct diagnose of UC and CD patients (Table 2). However, until now none of the identified biomarkers have been implemented in daily use^[15].

The first group to publish a discovery-based proteomics study of the IBDs was Barceló-Batllori *et al.*^[66] in 2002. The aim of the study was to identify potential cytokine regulated proteins in colon epithelial cells isolated from IBD patients, which might be involved in the pathogenesis of IBDs. Human adenocarcinoma cells were *in vitro* exposed to known cytokines expressed in IBD, namely interferon-gamma, IL-1-beta and IL-6 (TNF-alpha was excluded as it is known to induce apoptosis in such cells). Using proteomics, the protein profiles of the cells were analyzed before and after exposure to the cytokines. All proteins from the cells were first separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). By staining all protein in the gels, different samples (gels) can be compared in terms of protein abundance based on the staining intensities, and differentiating protein spots can be visually identified. Spots of interest were cut from the gel with a knife and the proteins were enzymatically digested to specific peptides using the protease trypsin (in-gel digestion). The digestion of proteins is an essential step for protein identification, as no MS technique currently exist that can identify thousands of intact proteins in a complex sample in a high throughput manner. This is only possible when using digested proteins (peptides). The proteins were identified based on the peptides using MS, with the technique called matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS (Figure 2A). MALDI-TOF MS is a sensitive technique, but it involves placing a few drops of the sample on a plate which is left to dry prior to analysis. During analysis a laser is used to evaporate small spots from the dried droplet and ions in the produced gas are

analyzed by MS. In the study, several cytokine regulated proteins were identified. Subsequently, human epithelial cells were isolated from UC patients and CD patients. Based on the findings, the samples were analyzed for the enzyme indoleamine-2,3-dioxygenase using antibodies by western blotting. The group found an overabundance of the enzyme indoleamine-2,3-dioxygenase in CD and UC compared to normal mucosa, hypothesizing an involvement of the Kynurenine pathway of tryptophan metabolism in the IBDs. Indoleamine-2,3-dioxygenase activity has furthermore been found to be essential in dendritic cells to induce co-cultured T cell apoptosis^[66].

When analyzing protein spots cut from gels the MALDI-TOF MS method is applicable, but the analysis of an entire 2D-PAGE gel is unfeasible, due to the commonly several thousand detectable spots. The technique is therefore less suitable for high-throughput identification of many thousand proteins. Therefore, when analyzing digested 2D-PAGE gels one usually only investigates changing protein spots and omits any information regarding non-changing protein spots. Information regarding non-changing proteins might prove equally important as changing proteins for studies seeking to describe disease etiologies. However, for biomarker studies 2D-PAGE strategies represent a feasible and proven way of identifying biomarker candidates. MALDI-TOF MS can also be conducted using intact proteins without prior enzymatic protein digestion. A variant of MALDI-TOF MS is to spot the protein mixture on a modified surface, to which the intact proteins bind and subsequently the intact masses of the proteins can be obtained by MS. This technique is called surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS) (Figure 2A). However, when studying intact proteins using MALDI-TOF MS or SELDI-TOF MS, one usually does not obtain identification of the detected signals.

Electrospray ionization (ESI) remains the only MS technique for identifying and quantifying several thousands of proteins in a high-throughput manner (Figure 2B). ESI involves spraying the digested proteins directly into the MS. By incorporating liquid chromatography (LC) with columns prior to the ESI process, the peptides can be separated and sequentially eluted over several hours. This gives the MS systems enough time to analyze a large proportion of the eluted peptides which subsequently can be identified. In this way, large-scale proteomic studies can be performed in a high-throughput manner using ESI LC-MS. These studies yield (relative) quantitative information of thousands of identified proteins in a single experiment, and thus might provide better information for explaining disease etiologies. In 2004, Hardwidge *et al.*^[67] published such a study, which was the first large scale proteomic analysis of a human cellular response to a pathogen. Discovery-based proteomics was applied to investigate the protein profiles (cellular response) of human Caco-2 intestinal epithelia cells before and after infection with *E. coli*. The group did not work directly with IBD, but the results are applicable to the diseases,

Table 2 Proteomics biomarker candidate studies and main findings

Ref.	Sample	Analysis	Findings and perspectives
Barcelo-Batllo <i>et al</i> ^[66] , 2002	<i>In vitro</i> colon epithelial cells and purified epithelial cells from UC and CD patients	2D-PAGE protein quantitation, and in-gel digestion and MALDI-TOF MS and Western blot protein identification	The enzyme indoleamine-2,3-dioxygenase was more abundant in cells from CD and UC patients compared to normal mucosa. Tryptophan and arginine metabolism may play a role in the IBDs
Hardwidge <i>et al</i> ^[67] , 2004	Human Caco-2 intestinal epithelia cells before and after infection with <i>E. coli</i>	ESI LC-MS protein identification and quantitation, Western blot verification	125 proteins more abundant and 139 proteins less abundant after infection, some related to innate immune responses. These proteins might be relevant to look for in future biomarker studies
Hsieh <i>et al</i> ^[68] , 2006	Colonic biopsies from UC, nonspecific infectious colitis patients and controls	2D-PAGE protein quantitation, and in-gel digestion and MALDI-TOF MS protein identification	6 proteins were found to be more abundant in UC and 13 less abundant. The result indicates that mitochondrial dysfunction might be involved in UC the etiology. Four biomarker candidates were identified, however, they require validation
Shkoda <i>et al</i> ^[69] , 2007	Intestinal tissue cells purified from patients suffering from CD, UC, and colon cancer	2D-PAGE protein quantitation, and in-gel digestion and MALDI-TOF MS and Western blot identification	Proteins associated with signal transduction, stress response and energy metabolism were differently abundant in inflamed and non-inflamed tissue. 32% of the differentially regulated proteins were involved in energy metabolism
Meuwis <i>et al</i> ^[10] , 2007	Serum from UC and CD patients	SELDI-TOF MS m/z signal profiling, MALDI-TOF MS and Western blot protein identification	Successful in differentiating CD from UC patients with a sensitivity of 85% and a specificity of 95% from several m/z signals. Four biomarker candidates were identified, all known acute inflammatory markers, limiting the diagnostic value. However, the feasibility of serum biomarker studies was demonstrated
Nanni <i>et al</i> ^[71] , 2007	Serum from UC, CD patients and healthy controls	Solid-phase bulk protein extraction, MALDI-TOF MS signal profiling	Able to separate the three groups with 97% prediction results. The signals were not identified, but the feasibility of serum biomarker studies was demonstrated
Meuwis <i>et al</i> ^[70] , 2008	Serum from responding and non-responding CD patients to infliximab	SELDI-TOF MS signal profiling, MALDI-TOF MS, Western blot and ELISA protein identification	Able to predict responders with a sensitivity of 79% and a specificity of 80%. Increased amount of PF4 was associated with non-response to infliximab with MS but not ELISA, so usability of PF4 as a biomarker seems limited
Nanni <i>et al</i> ^[72] , 2009	Intestinal epithelial cells from CD patients and healthy controls	1D-PAGE and in-gel digestion, ESI LC-MS protein identification and quantitation	Proteins more abundant in CD patients include several proteins involved in inflammation processes, and less abundant include Annexin A1, involved in the anti-inflammatory action. Follow-up research is required to assess the feasibility of the biomarker candidates
Hatsugai <i>et al</i> ^[73] , 2010	Peripheral blood mononuclear cells from UC and CD patients, and healthy controls	2D-PAGE quantitation, and in-gel digestion and MALDI-TOF MS protein identification	Successfully discriminated UC from CD based on seven differently present proteins, all associated with inflammation oxidation/reduction, the cytoskeleton, endocytotic trafficking and transcription. The biomarker candidates require validation using a larger number of patients, but seems promising
M'Koma <i>et al</i> ^[74] , 2011	Mucosal and submucosal layers of samples originating from CC and UC patients	MALDI-TOF MS m/z signal characterization, no protein identification	Five m/z signals were detected in the submucosal layer, which could separate the two groups with an accuracy of 75 percent. The signals needs to be identified, however, the disease groups can be separated on basis of the mucosal and submucosal profiles
Presley <i>et al</i> ^[75] , 2012	Microbes and human proteins at the intestinal mucosal-luminal interface from CD and UC patients, and healthy controls	Oligonucleotide ribosomal RNA fingerprinting, SELDI-TOF MS and MALDI-TOF MS identification	35% of the detected bacterial phylotypes were present in different amounts in the diseases, indicating the involvement of host-microbe interactions in IBD. The microbiome might prove useful as a target for therapy
Han <i>et al</i> ^[14] , 2013	Colonic tissue biopsies of Korean IBD patients	ESI LC-MS protein identification with label-free quantitation	27 potential biomarkers were identified for UC, 37 biomarkers for CD and 11 proteins commonly associated with IBD. Three novel biomarkers were identified for active CD: Bone marrow proteoglycan, L-plastin and proteasome activator subunit 1. The biomarker candidates require validation, but might prove feasible as new diagnostic and therapeutic targets
Seeley <i>et al</i> ^[76] , 2013	Histological tissue layers from UC and CC patients	MALDI-TOF MS m/z signal characterization, no protein identification	114 different m/z signals were found to be different between the two groups. The signals remain unidentified
Gazouli <i>et al</i> ^[77] , 2013	Serum samples from responding and non-responding CD patients to infliximab treatment	2D-PAGE quantitation, and in-gel digestion and MALDI-TOF MS protein identification	15 differently abundant proteins between responders and non-responders to infliximab were identified. The biomarker candidates require further validation

IBD: Inflammatory bowel diseases; UC: Ulcerative colitis; CD: Crohn's disease; MALDI-TOF MS: Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry; 2D-PAGE: Two-dimensional polyacrylamide gel electrophoresis; ESI: Electrospray ionization; SELDI-TOF MS: Surface-enhanced laser desorption/ionization time of flight mass spectrometry; LC: Liquid chromatography.

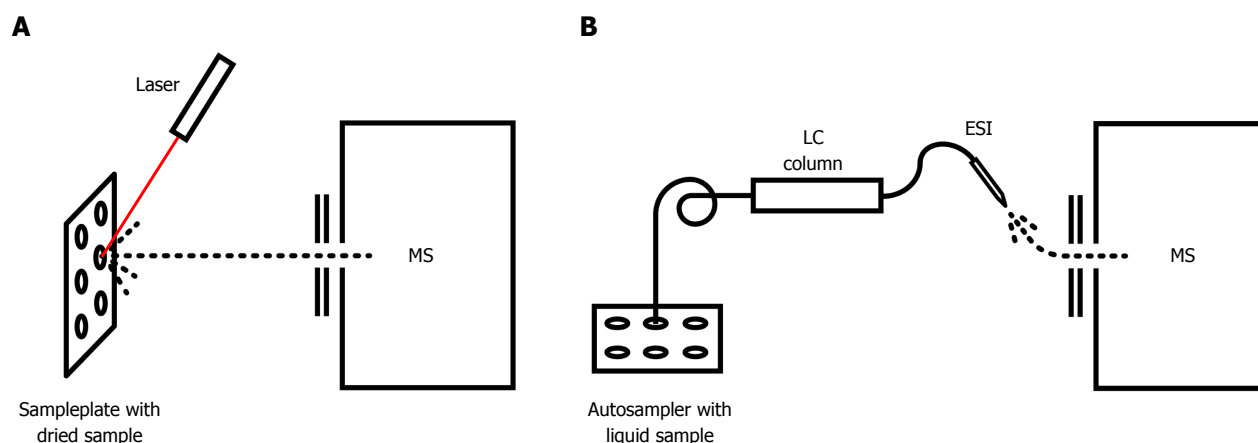


Figure 2 Two commonly used mass spectrometry techniques. A: Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MS), where the peptide or protein sample is dried on a target plate. Subsequently, a laser is used to evaporate the dried sample, and the generated gas phase ions are analyzed by the mass spectrometer; B: Liquid chromatography (LC)-electrospray ionization (ESI) MS, where the liquid peptide (or protein) sample is separated on a LC column, and sequentially eluted often over several hours. The eluted peptides are injected directly into the mass spectrometer by ESI and analyzed.

as the involvement of host-microbe interactions in the IBDs have been suggested^[54]. The cells were lysed, and the lysates were chemically modified using chemical labels to allow for a relative comparison between the protein abundances measured by MS. Using ESI LC-MS, the group recorded 10921 peptide fragments mass spectra, from which they were able to identify 2000 proteins. Two hundred and sixty four proteins had a known biological function and were found to have at least a 2-fold abundance difference between infected and non-infected, roughly half were more abundant post infection. Some of the MS-findings were verified with western blots, and significant changes were found in amount of actin-related proteins before and after infection.

Even though ESI LC-MS has advantages in terms of high-throughput, many biomarker studies have successfully employed MALDI-TOF MS protein identification in IBDs. In 2006, Hsieh *et al*^[68] applied discovery based proteomics using such a platform. The group analyzed the etiology and pathogenesis of UC using colonic biopsies to detect any significant difference in the protein profiles. The biopsies were obtained from four UC patients, three patients with nonspecific infectious colitis and five individuals with no obvious colonic disease. The proteins were separated by 2D-PAGE and a total of 1000 protein spots were compared visually between the diseased *vs* normal colon mucosa tissues. Forty protein-spots were found to be consistently different in intensity. Spots of interest were cut from the gel, tryptic digestion was performed and 19 proteins were identified using MALDI-TOF MS. Hereof, 13 identified proteins were less abundant in the UC-group and six proteins were more abundant. Eight of the less abundant proteins were identified as being mitochondrial proteins, suggesting that mitochondrial dysfunction might be involved in UC.

A year later in 2007, Shkoda *et al*^[69] also identified a potential association between dysfunction in the en-

ergy metabolism and IBDs. The group applied a similar strategy and platform to investigate the loss of intestinal cell function, a critical component in the initiation and perturbation of chronic intestinal inflammation, and was the first to compare inflamed and non-inflamed tissue from the same patient. Intestinal cells were purified from intestinal tissue obtained from patients suffering from CD, UC, and colon cancer. The proteins were separated by 2D-PAGE and analyzed by MALDI-TOF MS and western blotting. 41 proteins were found to be differently abundant between inflamed and non-inflamed tissue, including proteins associated with signal transduction, stress response and energy metabolism. Thirty-two percent of all detected differentially regulated proteins associated with IBD were involved in energy metabolism. In 2007, Meuwis *et al*^[70] published the first proteomic serum profiling study using SELDI-TOF MS in IBD, a variation of MALDI-TOF MS. The study included 30 patients with CD, 30 patients with UC, 30 inflammatory controls and 30 healthy controls. By characterizing the serum only by the *m/z* signals and not identified proteins with SELDI-TOF MS, the group was able to differentiate CD from UC with sensitivity of 85% (51/60) and specificity of 95% (57/60). Several of the unidentified signals were subsequently identified by MALDI-TOF MS, western blotting, and ELISA assay. Four biomarker candidates were identified: platelet aggregation factor 4 (PF4), myeloid related protein 8, fibrinopeptide A and haptoglobin alpha-2 subunit. All four proteins are known acute inflammatory markers to be expected in the IBDs, but the study succeeded in demonstrating that the separation of CD and UC patients based on serum markers is possible, highlighting the potential of serum profiling.

A year later, Meuwis *et al*^[70] used the same platform and strategy to analyze if serum from 20 CD patients could be used to predict response to infliximab treatment. Infliximab is a monoclonal antibody against TNF-alpha, and was the first anti-TNF-alpha agent accepted for

IBD treatment. The protein profiles were characterized in serum prior to and post treatment with SELDI-TOF MS. The group verified the four previous biomarkers, and especially increased amount of PF4 was associated with non-response to infliximab. However, the association could not be confirmed by ELISA, and did not correlate significantly with other disease markers. Even so, the study was able to predict responders with a sensitivity of 79% (55/70) and a specificity of 80% (56/70). Even though the study did not succeed in identifying a usable biomarker for the prediction of responders, the study highlighted the potential in proteomic studies and response marker discovery.

In 2007, Nanni *et al.*^[71] optimized the methodological approach used to evaluate serum with MALDI-TOF MS. Using a solid-phase bulk protein extraction protocol followed by MALDI-TOF MS, they analyzed serum from 15 CD, 26 UC and 22 healthy individuals and were able to separate the three groups with 97% prediction results. Two years later, Nanni *et al.*^[72] conducted a study using high-throughput ESI LC-MS to investigate protein variations in the intestinal epithelial cells from CD patients. However, in contrast to Hardwidge *et al.*^[67] in 2004 who used chemical labelling of the peptides to measure the relative abundances, Nanni *et al.*^[72] employed a label-free strategy, and relied on the accurate detection of the peptide masses. In this way, significant savings can be achieved for large studies and the sample preparation protocols simplified. Intestinal epithelial cells were isolated from samples originating from two CD patients and two control patients. The cells were lysed and the proteins were separated by 1D-PAGE, where the proteins are separated only in one dimension in contrast to 2D-PAGE, which allowed the entire visualized gel lane to be cut into pieces and digested with trypsin. The resulting peptides were analyzed by ESI LC-MS and by comparing the peptide intensities, relative protein abundances could be calculated. Proteins which were found to be more abundant in the epithelial cells from CD patients include heat shock protein 70, tryptase alpha-1 precursor as well as several proteins involved in inflammation processes. The nuclear protein Annexin A1, involved in the anti-inflammatory action, and the malate dehydrogenase enzyme was found to be less abundant. The feasibility of the biomarker candidates remains to be validated. However, of great importance is the demonstration of the utility of label-free ESI LC-MS analysis for the identification of differences in protein abundances for IBD.

In 2010, Hatsugai *et al.*^[73] performed the first study which successfully discriminated UC from CD completely. The group analyzed peripheral blood mononuclear cells from 17 UC patients, 13 CD patients and 17 healthy controls. The proteins were separated by 2D-PAGE and more than 1000 protein spots were detected in each gel. Five hundred and forty-seven protein spots were selected for the quantitative analysis, and 34 protein spots were significantly different between the UC and CD groups. Using 58 protein spots, the UC and CD patients could

be differentiated. The 58 protein spots were furthermore subjected to in-gel tryptic digestion followed by MALDI-TOF MS protein identification. Eleven of the proteins were successfully identified, and were found to be functionally related to inflammation, oxidation/reduction, the cytoskeleton, endocytotic trafficking and transcription. The profiles could, furthermore, predict disease severity and the UC patients' responses to treatment.

In 2011, M'Koma *et al.*^[74] analyzed mucosal and sub-mucosal layers of samples originating from Crohn's colitis (CC) and UC, using MALDI-TOF MS. Five unknown m/z MS signals were detected, which could separate the two groups. The study did not identify the origin of the signals, but highlighted the possibility of finding biomarkers in the intestinal tissue.

As mentioned earlier, even though we are far from having a complete picture of the intestinal micro-biome, changes in the bacterial composition have been detected in IBD. In 2012, Presley *et al.*^[75] investigated the host-microbe interaction at the intestinal mucosal-luminal interface of 14 CD patients, 21 UC, and 16 healthy controls. The mucosa prevents microorganisms from entering the host tissue. Using a novel saline-lavage technique, saline was injected during colonoscopy and extracted again to avoid interference from the intestinal layer contents resulting from a biopsy sample. The bacterial ribosomal RNA genes were analyzed by oligonucleotide fingerprinting and the proteins were analyzed by SELDI-TOF MS and MALDI-TOF MS. A combined proteome was constructed, constituting the proteomes from all detected organisms. Of the 3374 detected bacterial phylotypes, 35% significantly differentiated the diseases, indicating that host-microbe interactions might be involved in IBD, presenting new possibilities for diagnosis and therapy.

In 2013, Han *et al.*^[14] analyzed colonic tissue of Korean IBD patients in a high-throughput manner using ESI LC-MS and label-free quantitation. The study included four UC patients, three CD patients and two with inflammatory related polyps related to UC. The biopsies were homogenized and digested with trypsin without prior prefractionation and on average 324 proteins were identified for each group. Even though the number of identified proteins is relatively low considering the 2000 proteins Hardwidge *et al.*^[67] identified in 2004, 27 potential biomarkers were identified for UC, 37 biomarkers for CD and finally 11 proteins that were commonly associated with IBD. Three novel proteins, bone marrow proteoglycan, L-plastin and proteasome activator subunit 1 were identified as potential biomarkers for active CD. These biomarkers need validation, however, the feasibility of conducting high-throughput proteomics with label-free strategies in biomarker discovery was demonstrated.

A study published in 2013 by Seeley *et al.*^[76] investigated histological layers of 62 confirmed UC and CC tissues by MALDI-TOF MS. A total of 114 m/z MS signals were found to be statistically different between the two groups, however the signals have yet to be identified.

Finally, in 2013, Gazouli *et al.*^[77] published a study

where the response of 18 CD patients to infliximab treatment was correlated with known serum biomarkers. Serum samples were analyzed using 2D-PAGE, and 240 protein spot were selected for in-gel digestion and subsequent MALDI-TOF MS protein identification. The group was successful in identifying 15 proteins which were differentially present in the serum of CD patients depending on the response to infliximab. The proteins apolipoprotein A-I, apolipoprotein E, basic complement C4, plasminogen, serotransferrin, beta-2-glycoprotein 1, and clusterin were found to be more abundant in the patient groups with clinical and serological non-responders and responders, than in the group of patients with clinical and serological remission. Additionally, leucine-rich alpha-2-glycoprotein, vitamin D-binding protein, alpha-1B-glycoprotein and complement C1r subcomponent were found to be more abundant in the serum of the group of patients with remission. Interestingly, the group was unable to confirm the findings by Meuwis *et al.*^[70], that PF4 could be a biomarker for infliximab response, emphasizing that the biomarker candidates need further validation. Nonetheless, the study was successful in demonstrating the feasibility of identifying biomarkers in the serum usable to predict treatment outcome.

As apparent, many studies have successfully applied proteomic strategies to identify biomarkers, investigate IBD pathogenesis and identify prognostic markers in serum, stools, and tissue. Several biomarkers have been found (Table 2), most related to unspecific inflammation, and all biomarker candidates identified so far lacks follow-up validation studies. However, even though many of the identified biomarkers are related to inflammation, the studies have demonstrated the feasibility and potential of the proteomics platform in IBD, and given clues to the mechanisms of the IBDs. A few studies have successfully differentiated CD patients from UC patients. However, only based on unidentified m/z signals and not using identified protein or peptide biomarkers, from which the disease etiologies might be better explained. Nonetheless, these studies demonstrate the presence of usable biomarkers yet to be identified. Identified biomarkers hold the potential for designing diagnostic ELISA tests and protein array chips, where antibodies are used to detect the abundance of one or more antigens^[78,79]. Such arrays could constitute new clinical tools for diagnosis, prognosis and identify novel targets for therapy.

The studies have demonstrated the presence of biomarkers, in serum, in the intestinal tissue and in stools. Many studies have aimed at performing global discovery-based proteomics in the intestinal tissue, and it has been demonstrated that high-throughput techniques such as ESI LC-MS, employing labelling or label-free quantitation are feasible ways to identify biomarkers in highly complex samples. The advantage of high-throughput protein identification and quantification strategies are especially apparent when disease etiologies are to be examined.

Furthermore, few studies have investigated the possible association between various PTMs and the IBD dis-

ease etiologies. Such an association is known from other inflammatory diseases; an example being the inflammatory joint-disease rheumatoid arthritis (RA) where the PTM citrullination is known to be involved in the etiology^[80-83].

POSTTRANSLATIONAL MODIFICATIONS AS BIOMARKERS

Today, more than 200 distinct PTM's are known^[84]. The PTMs are, to a large extend, important for the physiological function of the protein and the half-life of PTMs range from milliseconds to years^[85]. Unfortunately, they are also often low abundance, highly diverse and complex, and thus can be challenging to detect and characterize^[25,27,86]. Hence, PTMs represent promising targets for biomarker discovery studies. For a review on protein regulation by PTMs in the IBDs, we refer to the work by Ehrentaut *et al.*^[5]. Common *in vivo* PTMs include phosphorylation, which is a reversible modification of the amino acids tyrosine, serine and threonine. Phosphorylation is known to be involved in activation and inactivation of enzyme activity, modulation of molecular interactions and cell signaling through specific domains. Acetylation can target any N-terminal, and it is believed that 84% of all human proteins undergo this modification^[87]. The PTM affects the protein stability, and histone acetylation is known to play a role in gene regulation. Glycosylation is another central PTM. It is reversible and known to be involved in cell-cell recognition and signaling, and regulation of proteins. Disulfide bond formation between two cysteines is a key element in the stabilization of proteins and protein complexes, such as, antibodies by forming intra- and intermolecular crosslinks. Deamidation of asparagine or glutamine is a possible regulator of protein-ligand and protein-protein interactions, and ubiquitination is a marker for protein recycling/destruction^[25]. Several PTMs are known to be involved in the inflammatory responses, and PTMs could be involved in the IBD disease etiologies. Lastly, citrullination is the irreversible deimination of arginine into citrulline, *in vivo* catalyzed by the peptidylarginine deiminases, a calcium binding family of enzymes^[88,89]. The exact role of the modification remains largely unknown, but the modification is believed to alter the fold of the proteins, change the protein polarity, and/or lead to denaturation in order to render the protein more prone to enzymatic degradation^[80,88,89]. Citrullination has been associated with several diseases, including Alzheimer's disease^[90], and RA where an anti-citrullinated protein antibody was identified^[80-83]. Smoking has been associated with increased citrullination, and smoking is the best known environmental factor for the development of RA^[91-95]. Several studies have, furthermore, associated smoking with an increased risk of developing CD and UC^[96-100]. In RA, it is believed that citrullination of proteins results in the generation of new antigens being presented to the immune system, which in turn triggers an autoimmune response^[83]. It therefore seems plausible that citrullination may have a similar role

in the IBDs as well as other inflammatory diseases. However, as with many PTMs the MS-driven detection of citrullinated proteins in a high-throughput manner is not straight forward^[84,101-104]. Nonetheless, if disease-specific citrullinated proteins could be identified, these could be utilized in ELISA or protein array chips for prognostics and/or diagnostics. An example of the utilization of a similar biomarker is the diagnosis of RA patients, where the presence of anti-citrullinated protein antibodies in the serum is used to detect the disease with a sensitivity of 71% and specificity of 95%^[80-83].

CONCLUSION

The diagnosis of UC and CD patients remains difficult, especially in the early stages of the diseases, and early and accurate diagnosis of IBD-patients is crucial. Several studies have successfully identified promising biomarkers in stools, serum and tissue, demonstrating the presence of IBD biomarkers. However, none of the identified biomarkers have been implemented in clinical daily use, and the diagnosis is based on a combination of disease history, colonoscopy inflammation biomarkers and histological evaluation.

Few studies have aimed at investigating the global proteome of intestinal tissue using high-throughput techniques such as ESI LC-MS, and the potential of such analysis seems immense. The recent development within the field of high-throughput protein identification using MS, now allows for identifying and quantifying several thousand proteins in a few hours of analysis time. Besides protein abundances, PTMs represent promising targets for biomarker discovery studies. An analysis of tissue, serum or stools therefore seems promising to identify novel biomarkers. Such information could be used to make accurate diagnostic and prognostic tools to differentiate patient groups and predict treatment responses. Antibodies against one or more identified diagnostic targets could be used in ELISA or protein array chips, which in turn can be used to detect the abundance of the given antigen. Besides aiding physicians in making a correct diagnosis and treatment strategy, knowledge of disease specific proteins and PTMs might identify disease pathways and new targets for therapeutic agents, leading to improved pharmaceutical drugs.

Conclusively, protein identification and quantification using mass spectrometry holds great promise for the identification of novel diagnostic and prognostic biomarkers for the IBDs, and might help explain the disease etiologies, ultimately leading to improved treatment strategies.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Inflammatory bowel disease of primary sclerosing cholangitis: A distinct entity?

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Abstract

This is a review of the characteristic findings of inflammatory bowel disease (IBD) associated with primary sclerosing cholangitis (PSC) and their usefulness in the diagnosis of sclerosing cholangitis. PSC is a chronic inflammatory disease characterized by idiopathic fibrous obstruction and is frequently associated with IBD. IBD-associated with PSC (PSC-IBD) shows an increased incidence of pancolitis, mild symptoms, and colorectal malignancy. Although an increased incidence of pancolitis is a characteristic finding, some cases are endoscopically diagnosed as right-sided ulcerative colitis. Pathological studies have revealed that inflammation occurs more frequently in the right colon than the left colon. The frequency of rectal sparing and backwash ileitis should be investigated in a future study based on the same definition.

The cholangiographic findings of immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) are similar to those of PSC. The rare association between IBD and IgG4-SC and the unique characteristics of PSC-IBD are useful findings for distinguishing PSC from IgG4-SC.

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Key words: Primary sclerosing cholangitis; Primary sclerosing cholangitis; Inflammatory bowel disease; Inflammatory bowel disease-associated with primary sclerosing cholangitis; Immunoglobulin G4-related sclerosing cholangitis

Core tip: Inflammatory bowel disease (IBD)-associated with primary sclerosing cholangitis (PSC) (PSC-IBD) shows an increased incidence of pancolitis, mild symptoms, and colorectal malignancy. Although an increased incidence of pancolitis is a characteristic finding, some cases are endoscopically diagnosed as right-sided ulcerative colitis. Pathological studies have revealed that inflammation occurs more frequently in the right colon than the left colon. The cholangiographic findings of immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) are similar to those of PSC. The rare association between IBD and IgG4-SC and the unique characteristics of PSC-IBD are useful findings for distinguishing PSC from IgG4-SC.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease characterized by idiopathic fibrous obstruction^[1]. The fibrosis causes diffuse narrowing of the intrahepatic and extrahepatic bile ducts, and the resulting persistent biliary stasis leads to hepatic cirrhosis and a poor prognosis. Liver transplantation is indicated as a treatment for PSC. According to the diagnostic criteria for PSC proposed by the Mayo Clinic in 1999^[1] and 2003^[2], in addition to cholangiographic findings, the presence of inflammatory bowel disease (IBD) is important.

However, several related studies with contradictory results have recently been published^[3,4].

IgG4-related sclerosing cholangitis (IgG4-SC) has recently been established as a new clinical entity^[5]. The cholangiograms of IgG4-SC are occasionally similar to those of PSC^[6]. The differential diagnosis of PSC and IgG4-SC is important because IgG4-SC patients respond well to steroid therapy^[7].

In this study, we aimed to clarify the clinicopathological characteristics of PSC-IBD and the usefulness of PSC-IBD in the diagnosis of SC.

LITERATURE SEARCH

We conducted a literature search of English articles related to PSC-IBD, published between 2005 and March 2013, using the following keywords: “PSC,” “primary sclerosing cholangitis,” “PSC-IBD,” “IBD,” “immunoglobulin G4 (IgG4)-related sclerosing cholangitis (IgG4-SC),” “autoimmune pancreatitis,” and “IgG4-related disease (IgG4RD).” We connected the key words using “OR”. Pertinent articles obtained from the literature search were reviewed. All references were manually verified, and all reference lists in the retrieved articles were scrutinized to identify any additional articles that might have been missed in the PubMed search. As clinical data on PSC-IBD are limited, the authors also considered their own > 20-year clinical experience in the treatment of PSC-IBD. This study was primarily limited to adults patients, with the exception of the “frequency of PSC-IBD” section.

FREQUENCY OF PSC-IBD

PSC is strongly associated with IBD, and the prevalence of PSC-IBD is as high as 60%-80% in western countries^[8]. Approximately 80% of IBD is represented by ulcerative colitis (UC), 10% by Crohn's disease (CD), and 10% by indeterminate colitis^[9]. Conversely, only 2%-7.5% of IBD patients develop PSC^[8].

Previous reports from Europe and the United States have indicated that IBD complicates a high proportion of PSC cases. In Japan, IBD is found in only 21%-32% of PSC cases, according to surveys conducted by the

Japanese Society of Gastroenterology^[10] and the Japan Society of Hepatology^[11]. In a second nationwide analysis, only 125 of 388 patients (32%) had an established diagnosis of IBD, 79% of whom had UC, whereas only 6.4% were diagnosed with CD^[11]. Ang *et al.*^[12] also reported a low association rate of 20% (2/10) between PSC and IBD in Singapore. The association of PSC with IBD varies depending on geographical location, with higher rates in European and American populations, and a significantly lower association in Asian patients^[13]. However, the incidence of IBD in PSC patients in our series was higher (68.9%) than that already reported in Japan^[14]. We speculate that this high incidence was noted because only PSC patients who had undergone total colonoscopy at clinical onset were enrolled in our study. Yamagishi *et al.*^[15] also reported a higher incidence of IBD in PSC patients (93%) examined by colonoscopy. It is possible that we overlooked the endoscopic findings, as we did not perform a careful total colonoscopy because the symptoms of IBD are mild and the endoscopic findings of the colon show only slightly abnormal changes. The second national survey in Japan also reported that the incidence of IBD in PSC patients increased to 61% when only PSC patients examined by total colonoscopy were enrolled^[11]. Therefore, the frequency of PSC-IBD should be evaluated among patients undergoing careful total colonoscopy. This selection criterion might decrease the observed differences in frequency between eastern and western countries.

The age at clinical onset of IBD is controversial and has not been clarified. Loftus *et al.*^[9] reported that the mean age of IBD diagnosis was higher among PSC-IBD patients (32 years) compared with controls (28 years). In contrast, Brackmann *et al.*^[16] reported that IBD patients with PSC were significantly younger at the onset of IBD symptoms (PSC: 19 years *vs* no PSC: 29 years; $P = 0.04$), whereas the colitis-colorectal cancer interval was similar to that of IBD patients without PSC (17 years *vs* 20 years; $P = 0.236$). Joo *et al.*^[4] showed that PSC patients with UC presented with UC at a significantly earlier age (mean age: 24.5 years) compared with UC controls (mean age: 33.8 years).

Takikawa *et al.*^[10,11] discovered two peaks in the PSC age distribution, which has never been observed in other countries, and revealed that most Japanese PSC patients associated with IBD were adolescents or young adults. A recent study from Canada also reported two peaks in the PSC age distribution^[17]. Our previous study also showed a two-peaked age distribution, and that patients with PSC-IBD were significantly younger than PSC patients without IBD (33.6 years *vs* 58.9 years, $P < 0.001$)^[14].

Although the diagnosis of IBD precedes that of PSC in most patients, a recent study found that a shift in the timing of diagnosis of the two diseases has occurred in recent years, with PSC more often being diagnosed first. PSC was diagnosed before IBD in a recent

Table 1 character of inflammatory bowel disease-associated with primary sclerosing cholangitis

Ref./Nation	Year	Endoscopic/histological findings			Histological findings
		Extension of IBD	Backwash-ileitis	Rectal sparing	
Loftus <i>et al</i> ^[9]	2005	Total colitis 56/61 (92% vs CUC 54%)	19/37 (51% vs CUC 7%)	32/61 (52% vs CUC 6%)	
United States			by endoscopy	by endoscopy	
Joo <i>et al</i> ^[4]	2009	Total colitis 34/40 (85% vs CUC 45%)	10/24 (35.7% vs 26.9%)	11/40 (27.5% vs 25%)	
United States			by histology	by histology	
Sano <i>et al</i> ^[14]	2010	Total colitis 6/20 (35% vs CUC 35%)	Not studied	Not studied	Significantly higher inflammation in the right colon
Japan		Right-sided 11/20 (55% vs 3.3%)			
		Left-sided 1/20 (5% vs 31.7%)			
Jørgensen <i>et al</i> ^[24]	2012	Total colitis 60/110 (55%)	11/93 (12%) by endoscopy	73/110 (66%) by endoscopy	Significantly higher inflammation in the right colon
Norway		Right-sided 25/110 (23%)	17/87 (20%) by histology	70/107 (65%) by histology	
		Left-sided 3/110 (3%)			
Boonstra <i>et al</i> ^[3]	2012	Total colitis 207/380 (83%)	< 10%	< 10%	
The Netherlands		Left-sided 9/380 (4%)			
Schaeffer <i>et al</i> ^[27]	2013	Total colitis (IBD preceding PSC)			
Canada		Right-sided (PSC following IBD)			

CUC: Chronic ulcerative colitis; IBD: Inflammatory bowel disease; PSC: Primary sclerosing cholangitis.

cohort (2003-2007) when compared with a early cohort (1993-1997) (50% vs 35%, $P = 0.0009$)^[18].

MECHANISM OF PSC-IBD PATHOGENESIS

Several important observations, coupled with the strong association between certain human leukocyte antigen (HLA) haplotypes and the frequency of concurrent extrahepatic autoimmune disorders, support the concept that PSC is an immune-mediated phenomenon^[19,20]. Three UC susceptibility loci to be associated with PSC, harboring the putative candidate genes REL, IL2 and CARD9 were identified^[19]. A recent study reported 12 significant genome-wide associations outside the HLA region, 9 of which were new, increasing the number of known PSC risk loci to 16. Despite comorbidity with IBD in 72% of the included cases, 6 of the 12 loci showed significantly stronger associations with PSC than with IBD, suggesting overlapping yet distinct genetic mechanisms for these two diseases^[20].

The pathogenesis of PSC has been elucidated from the standpoint of PSC-IBD. Translocation of microbial flora across an inflamed, permeable gut with subsequent activation of the immune system and inflammation of the biliary tree is a hypothesized mechanism for the development of PSC. Activated intestinal lymphocytes enter the enterohepatic circulation and persist as memory cells that cause hepatic inflammation. Chemokines and adhesion molecules shared by the intestine and liver could contribute to immune cell binding at both sites^[21]. The observations that PSC can develop after colectomy^[22] and that IBD can develop after liver transplantation^[23] have led some investigators to suggest that aberrant homing of lymphocytes between the intestine and liver could be involved in the pathogenesis of PSC^[21]. Three recent studies indirectly supporting this theory have been published. Patients who received a liver trans-

plant had lower clinical and histological IBD activity than the non-transplant group^[24]. Marelli *et al*^[25] reported that progressive PSC requiring liver transplantation is associated with a milder course of UC, including reduced disease activity and less use of steroids, azathioprine, and surgery. Navaneethan *et al*^[26] reported that severe, progressive PSC requiring liver transplantation appeared to reduce the disease activity of UC and the need for colectomy.

However, these theories cannot explain why only 2%-7.5% of IBD patients develop PSC^[8], whereas PSC is strongly associated with IBD, or why CD is less associated with PSC. It is also unclear why immunosuppression does not improve PSC.

CLINICOPATHOLOGIC CHARACTERISTICS OF PSC-IBD

Previous studies have suggested that PSC-IBD differs from IBD without PSC in several aspects. Table 1 summarizes the reports concerning PSC-IBD^[3,4,9,14,24,27]. PSC-IBD has been reported to show an increased incidence of pancolitis, rectal sparing, backwash ileitis, mild symptoms, and colorectal malignancy^[8]. However, these results are controversial. All of the investigators agree that inflammation involved in PSC-IBD is milder than that in typical UC without PSC. Moreover, the majority of PSC-IBD patients show no or few IBD symptoms. Moayyeri *et al*^[28] reported that the number of hospitalizations and courses of steroid therapy decreased significantly in UC-PSC patients compared with UC controls. Our study also indicated that none of the enrolled patients had a severe clinical course, and that half of them were asymptomatic^[14].

However, varied findings concerning the extent of PSC-IBD have been reported. Although some authors have reported that pancolitis is characteristic of PSC-IBD^[3,4,9], others have insisted that right-sided IBD is

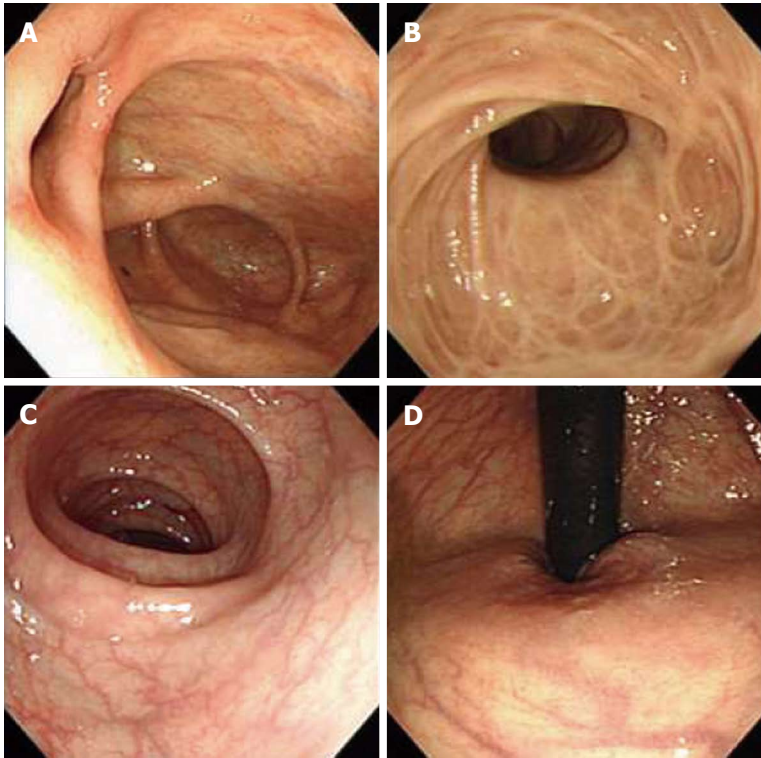


Figure 1 Colonoscopic findings at clinical onset. A: Cecum; B: Ascending colon; C: Sigmoid colon; D: Rectum. A 43-year-old female patient diagnosed with asymptomatic, concurrent primary sclerosing cholangitis-inflammatory bowel disease. The first colonoscopy showed multiple white scars in the ascending colon and right-sided transverse colon and no abnormal findings in the left-sided transverse colon, descending colon, sigmoid colon, or rectum.

characteristic^[14,24]. A recent report concluded that when IBD precedes PSC, pancolitis is common, whereas when PSC precedes IBD, right-sided IBD is common^[27].

Two reports revealed that the degree of inflammation was stronger in the right colon than the left colon using the histological studies. The first report demonstrated severe inflammatory cell infiltration in the cecum and ascending colon of PSC-IBD patients, whereas the degree was mild in the rectum/descending colon ($P = 0.0012$); goblet cell disappearance was also observed more frequently in the cecum/ascending colon than in the rectum/descending colon ($P < 0.05$)^[14]. The second report revealed that the histopathological signs of inflammation involved the right colon in 86% of patients and were purely right-sided in 23%. The frequency of inflammatory findings was higher in the right colon than the left colon ($P < 0.01$), but the general level of inflammatory activity was low^[24]. They also reported that inflammatory findings were more frequent on histology than on endoscopy.

These discrepancies may be due to differences in the geographical location between studies or the method used to assess total colitis based on the maximal extent of colitis at any time during the study period. Fluctuations in the extent of inflammation may therefore explain the high frequency of total colitis^[24,29].

The frequency of rectal sparing and backwash ileitis differs between reports, with the frequency of backwash

ileitis ranging from $< 10\%$ to 51% . Jørgensen *et al.*^[24] speculated that repeated examination or different patient selection criteria account for these differing results, and also reported that the frequency of rectal sparing on histological examination (20%) was higher than that on endoscopic examination (12%). Nakazawa *et al.*^[5] speculated that the different results were due to the definition and criteria of backwash ileitis.

The frequency of rectal sparing ranges from $< 10\%$ to 66% . Loftus *et al.*^[9] reported that rectal sparing was observed in 52% of UC-PSC patients compared with only 6% of UC patients without PSC on endoscopy. Jørgensen *et al.*^[24] reported that rectal sparing was observed in 66% of patients on endoscopy and was consistent with the results of pathological examination. In their study, the patients were considered to have rectal sparing if the inflammation involved the rectum but was less severe than that of the more proximal area of the colon. Joo *et al.*^[4] reported that rectal sparing was observed in only 27.5% of patients on histologic studies. Rectal sparing was defined as predilection if all biopsies from the rectum or the entire rectal mucosa from a resection specimen showed histologically normal mucosa. Rectal sparing was considered relative if at least one biopsy from the rectum at any time period or one portion of the rectal mucosa from the colonic resection specimen showed histologic features of chronicity, but at least mild activity was present in the areas of the mucosa proximal

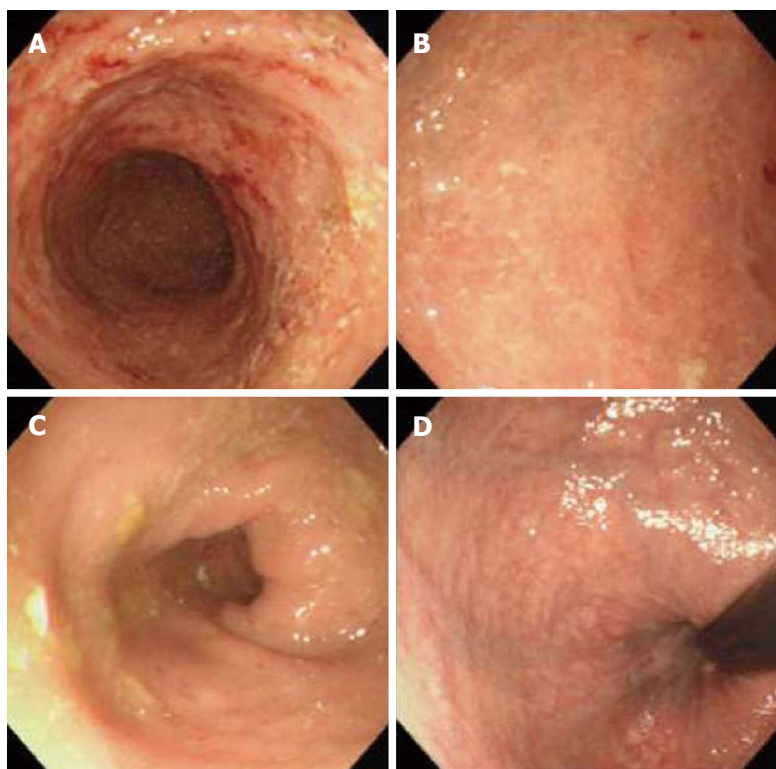


Figure 2 Colonoscopic findings seven months later. A: Cecum; B: Ascending colon; C: Sigmoid colon; D: Rectum. A repeat colonoscopy seven months later showing inflamed mucosa with multiple erosions and redness from the ascending colon to the right-sided transverse colon. Mucosal vessels are clearly visible in the descending colon, sigmoid colon, and rectum.

to the rectum.

The extent of colon involvement, rectal sparing, and backwash ileitis should be defined internationally to clarify the characteristics of PSC-IBD.

In our experiences with endoscopy, inflammation was more severe on the right, and rectal sparing appeared to be present on endoscopy (Figures 1 and 2), by contrast, less inflammatory cell infiltrations was observed in the rectum on histology. These findings are very useful in the diagnosis of PSC. In the presence of multiple biliary stenoses on magnetic resonance cholangiography, inflammatory findings dominant in the right colon and rectal sparing by total colonoscopy, invasive endoscopic retrograde cholangiography can be avoided in clinical practice.

COLORECTAL NEOPLASIA IN PSC-IBD PATIENTS

PSC-IBD patients are at particularly high risk for the development of colorectal cancer. Boonstra *et al*^[3] estimated that the colorectal cancer risk was increased 10-fold in PSC-IBD patients compared with UC controls. Claessen *et al*^[29] have reported that patients with PSC-IBD have a high long-term risk of developing colorectal cancer, and that this risk is approximately three-fold higher than that of cholangiocarcinoma. In patients with PSC-IBD, the 10-year and 20-year risks of colorectal cancer have

been reported to be 14% and 31%, respectively. Among 75 PSC-IBD patients, PSC was the only independent risk factor for the development of colorectal cancer, and the overall survival rate without liver transplantation was also reduced^[30].

PSC-IBD patients tend to be younger at colorectal cancer diagnosis^[31]. Colorectal cancer develops at a much younger age in these patients (39 years, range: 26-64 years) compared with IBD controls (59 years, range: 34-73) ($P = 0.019$)^[3].

Colorectal cancer in PSC-IBD patients predominantly develops in the right colon. The tumors were located proximally to the splenic flexure in 18 (67%) patients with PSC and in 52 (36%) patients without PSC ($P = 0.006$)^[29]. Thackeray *et al*^[32] also reported that colorectal cancer is most prevalent in the proximal colon (65%). When such patients are diagnosed with cancer, they tend to have more advanced tumors than IBD patients without concurrent PSC. In their study, patients with PSC had significantly more tumors with an American Joint Committee on Cancer tumor stage of 3A or higher when compared with patients with IBD alone (61.5% and 38.5%, $P = 0.003$). The reason for the preferential right-sided location of colorectal cancer in PSC patients remains unknown, although it has been speculated that the overall increased frequency of colorectal cancer in these patients could be due to a cytotoxic effect on the colonic mucosa caused by an altered bile acid composi-

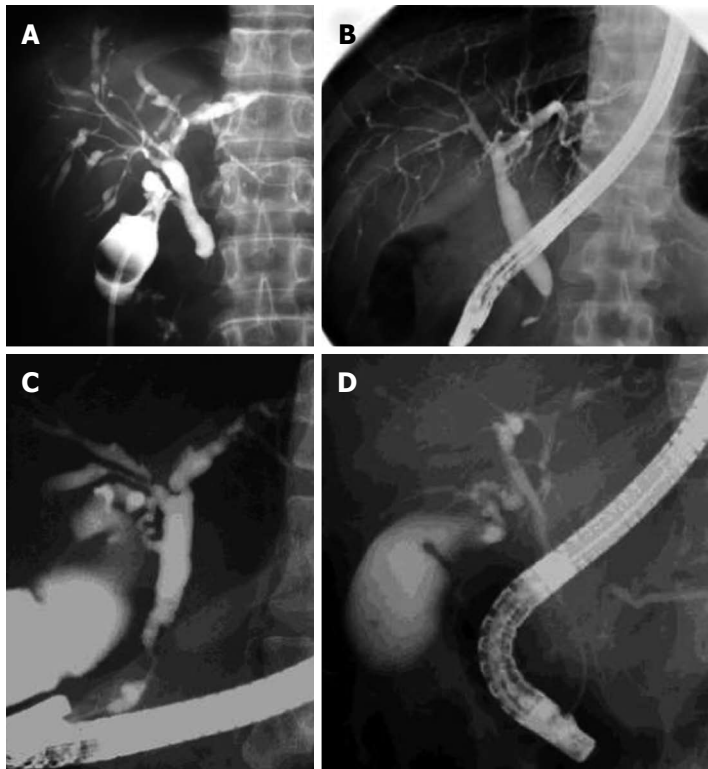


Figure 3 Cholangiographic examples of immunoglobulin G4-related sclerosing cholangitis and primary sclerosing cholangitis. Cholangiograms of immunoglobulin G4-related sclerosing cholangitis showing multiple stenoses in the intrahepatic ducts and stenosis in the intrapancreatic portion (A, B). Cholangiograms of primary sclerosing cholangitis showing a beaded appearance (C) and pruning of the intrahepatic ducts (C, D).

Table 2 Inflammatory bowel disease association in sclerosing cholangitis *n* (%)

Ref.	IgG4-SC	PSC	
Nishino <i>et al</i> ^[44]	0/24 (0)	15/24 (62.5)	$P < 0.0001$
Zen <i>et al</i> ^[45]	0/17 (0)	5/5 (100)	$P < 0.0001$
Ghazale <i>et al</i> ^[46]	3/53 (6)	70%	
Mendes <i>et al</i> ^[41]	NA	Elevated group (<i>n</i> = 12) 6/12 (50) Normal group (<i>n</i> = 115) 97/115 (90)	
Nakazawa <i>et al</i> ^[5]	0/62 (0)	21/31 (68)	$P < 0.0001$

IgG4-SC: Immunoglobulin G4-related sclerosing cholangitis; PSC: Primary sclerosing cholangitis.

tion. The predominance of right-sided inflammation is also a predisposing factor^[24]. The two reports showing that the histologic findings of inflammation were higher in the right compared to the left colon in PSC-IBD patients are consistent with the preferential right-sided location of colorectal cancer^[14,24].

Previous studies have shown conflicting results regarding the course of IBD after liver transplantation in patients with PSC. Recent studies have shown that the increased risk of neoplasia is maintained after liver transplant and proctocolectomy. Hanounch *et al*^[33] reported that patients with PSC-IBD after liver transplantation had a similar rate of colon neoplasia compared to those without liver transplantation (34% *vs* 30%, $P = 0.24$) during a mean follow-up period of 54.7 ± 47.7 mo. Jørgensen *et al*^[24] showed that macroscopic colonic

inflammation was more frequent after liver transplantation than before transplantation. The rate of relapse after transplantation was higher than that before transplantation, and the overall clinical IBD activity was also increased. Immunosuppression affects IBD activity after liver transplantation in patients with PSC.

Early cancer detection through enrollment in surveillance programs is the only strategy available to decrease cancer risk^[31]. More extensive colitis with a concurrent mild or even asymptomatic course, resulting in diagnostic delay and a lower colectomy rate, may contribute to an increased risk of colorectal cancer development^[9]. With the initial diagnosis of PSC in subjects with IBD, immediate and annual surveillance colonoscopy and biopsy analysis, of the entire colon, are recommended^[32,34].

The role of ursodeoxycholic acid (UDCA) as a chemopreventive agent is controversial. A meta-analysis showed no significant protective association between UDCA use and colorectal neoplasia. However, there was a significant chemopreventive effect on the risk of advanced colorectal neoplasia (OR = 0.35, 95%CI: 0.17-0.73), and low-dose UDCA use (8-15 mg/kg per day) was associated with a significantly reduced risk of colorectal neoplasia^[35]. There were no significant differences in cholangiocarcinoma incidence between the high-dose UDCA (17-23 mg/kg per day) and placebo groups^[36]. A recent study showed that patients treated with high-dose UDCA had a significantly higher risk of developing colorectal neoplasia during the study compared with those who received placebo (HR = 4.4, $P = 0.02$)^[37].

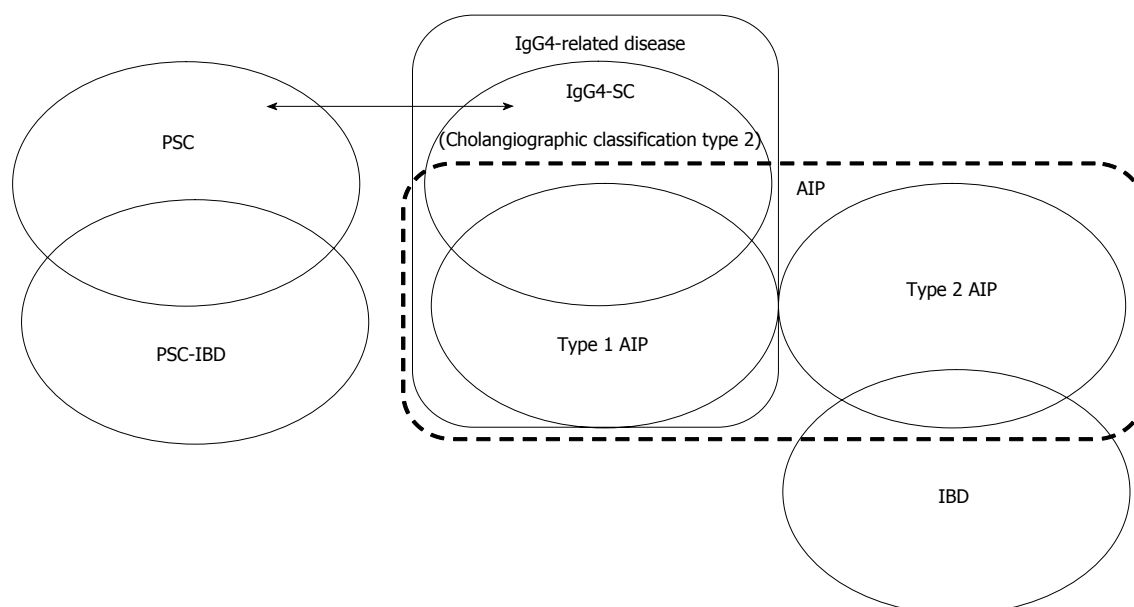


Figure 4 Correlation between inflammatory bowel disease and sclerosing cholangitis. PSC is frequently associated with characteristic PSC-IBD, whereas IgG4-SC is not associated with IBD. IgG4-SC is frequently associated with type 1 AIP, whereas type 2 AIP is frequently associated with IBD. PSC: Primary sclerosing cholangitis; PSC-IBD: IBD-associated with PSC; IBD: Inflammatory bowel disease; IgG4-SC: Immunoglobulin G4-related sclerosing cholangitis; AIP: Autoimmune pancreatitis.

THE USEFULNESS OF PSC-IBD CHARACTERISTICS IN THE DIAGNOSIS OF SCLEROSING CHOLANGITIS

Recently, IgG4-SC has attracted much attention following the emergence of clinical characteristics that distinguish it as a new clinical entity^[5]. IgG4-SC shows various cholangiographic features similar to those of pancreatic cancer, PSC, and cholangiocarcinoma^[7]. The characteristic cholangiographic features of IgG4-SC can be classified into four types based on the stricture location revealed by cholangiography and differential diagnosis^[6]. Type 2 IgG4-SC, in which stenosis is diffusely distributed throughout the intrahepatic and extrahepatic bile ducts, should be differentiated from PSC^[38] (Figure 3).

The differential diagnosis of PSC and IgG4-SC is important because patients with IgG4-SC show a good response to steroid therapy. We previously reported the differences between IgG4-SC and PSC^[7]. The age at clinical onset was significantly higher for IgG4-SC patients. Among the chief complaints in IgG4-SC, obstructive jaundice, reflecting marked concentric stenosis of the large bile duct, was most frequently observed. An elevated serum IgG4 level is a characteristic feature of IgG4-SC^[39]. An elevated serum IgG4 level and the association with type 1 autoimmune pancreatitis (AIP) are the most useful findings for discriminating between IgG4-SC and PSC^[6]. However, elevation of the serum IgG4 level alone is not useful because some PSC cases also show increased IgG4 levels. In addition, some IgG4-SC cases are not associated with AIP^[40]. Mendes *et al*^[41] measured the serum IgG4 levels in 127 patients with PSC and

found that it was elevated in 12 patients (9%). Björns-son *et al*^[42] reported elevated serum IgG4 levels in 12% of 285 patients with classic PSC. We performed a multicenter study in Japan to establish a cutoff value to differentiate IgG4-SC from controls^[43]. Serum IgG4 levels were compared between 56 patients with type 2 IgG4-SC and 110 patients with PSC. The serum IgG4 levels of the IgG4-SC patients were significantly higher than those of the PSC patients (799 ± 800 mg/dL *vs* 68.7 ± 86.0 mg/dL, respectively, $P < 0.001$). When we set the IgG4 cutoff value at 135 mg/dL, the sensitivity, specificity, and accuracy were 94.5%, 85.0%, and 90.5%, respectively. We also identified 13 of the 110 PSC patients (11.8%) with IgG4 values higher than the cutoff value of 135 mg/dL^[43]. Further studies are expected to clarify the differences between IgG4-SC and PSC patients with high serum IgG4 levels.

In contrast, the diagnosis of PSC is difficult because there are no useful markers of PSC. As such, an association with IBD is a very useful finding in the diagnosis of PSC. The frequencies of IBD associated with IgG4-SC are summarized in Table 2^[5,41,44-46]. No association between IBD and IgG4-SC has been reported, which is in contrast to the strong association between IBD and PSC (60%-80%)^[5,44-46]. One study reported that 6% of IgG4-SC cases were associated with IBD^[46], as IBD is common in western countries. We are unable to describe the details of IBD-associated IgG4-SC, although we speculate that the unique characteristics of PSC-IBD can be used to distinguish PSC from IgG4-SC.

IgG4-SC is frequently associated with type 1 AIP^[5]. Type 1 AIP is a different clinical entity from type 2 AIP, which is closely associated with IBD. Type 2 AIP is not

associated with IgG4-related diseases, including IgG4-SC, and elevated serum IgG4 levels are not observed^[47]. Clinically diagnosed IBD is incorporated in the diagnostic criteria for type 2 AIP in international consensus diagnostic criteria for autoimmune pancreatitis^[47]. These complicated associations are illustrated in Figure 4. There are no reports concerning the characteristics of IBD associated with type 2 AIP. If IBD associated with type 2 AIP shows characteristic findings, they might be useful not only in discriminating type 2 AIP from type 1 AIP, but also in elucidating the mechanism of sclerosing cholangitis.

In summary, PSC-IBD is associated with an increased incidence of pancolitis, mild symptoms and colorectal malignancy. Although an increased incidence of pancolitis is also a characteristic finding, some cases are endoscopically diagnosed as right-sided UC. Pathological studies have revealed that the findings of inflammation were more prevalent in the right colon than the left colon. The frequency of rectal sparing and backwash ileitis should be investigated in a future study based on the same definition. The rare association between IBD and IgG4-SC and the unique characteristics of PSC-IBD are useful for discriminating between PSC and IgG4-SC. In particular, when IBD is characterized by right-sided UC, rectal sparing, and backwash ileitis, the possible diagnosis of PSC-IBD should be considered. In the presence of multiple biliary stenoses on magnetic resonance cholangiography, inflammatory findings dominant in the right colon and rectal sparing by total colonoscopy, invasive endoscopic retrograde cholangiography can be avoided in clinical practice.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Dysregulation of mucosal immune response in pathogenesis of inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis. The exact etiology and pathology of IBD remain unknown. Available evidence suggests that an abnormal immune response against the microorganisms in the intestine is responsible for the disease in genetically susceptible individuals. Dysregulation of immune response in the intestine plays a critical role in the pathogenesis of IBD, involving a wide range of molecules including cytokines. On the other hand, besides T helper (Th) 1 and Th2 cell immune responses, other subsets of T cells, namely Th17 and regulatory T cells, are likely associated with disease progression. Studying the interactions between various constituents of the innate and adaptive immune systems will certainly open new horizons of the knowledge about the immunologic mechanisms in IBD.

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Key words: Crohn's disease; Inflammatory bowel disease; Proinflammatory cytokines; T helper cells; T

helper 17 cells; Ulcerative colitis

Core tip: The etiology and pathology of inflammatory bowel disease (IBD) remain elusive, and dysregulation of the mucosal immune response toward commensal bacterial flora together with genetic and environmental factors may play important roles in the pathogenesis of IBD. A better understanding of the mechanisms of immune responses in the intestinal mucosa will provide new insights into the pathogenesis of IBD, and shed some light on targeted immune therapy for this disease.

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INTRODUCTION

The etiology and pathogenesis of inflammatory bowel disease (IBD) remain elusive, and accumulating evidence has indicated that sustained intestinal infections, mucosal barrier defects, mucosal immune dysregulation, genetic and environmental factors are involved in the disease process^[1-4]. Among these, the dysfunction of the mucosal immune system plays an important role in the pathogenesis of IBD (Figure 1). Among a variety of inflammatory cells in the gut, mucosal CD4⁺ T cells are thought to play a central role in both the induction and persistence of chronic inflammation by producing proinflammatory cytokines. Previous studies have indicated that T helper (Th) 1-related cytokines [e.g., tumor necrosis factor (TNF), interferon (IFN)- γ , interleukin (IL)-12] as well as Th17-associated cytokines (e.g., IL-17A, IL-21, IL-23) are markedly increased in inflamed mucosa of Crohn's disease

(CD) patients, whereas the cytokine profiles in inflamed areas of ulcerative colitis (UC) patients seem to exhibit increased production of the Th2-associated cytokines such as IL-4 and IL-13^[1-3]. These proinflammatory cytokines are potent *in vitro* stimulators of intestinal mucosal effect or functions, including T cell and macrophage proliferation, adhesion molecule expression, chemokine expression, and secretion of other proinflammatory cytokines.

ABNORMAL IMMUNE RESPONSE IN THE INFLAMED MUCOSA OF IBD PATIENTS

Antigen-specific activation of various lymphocytes within the intestinal mucosa by enteric pathogens is an important feature of IBD immunopathology^[1-4]. Under physiological conditions, a large number of innate and immune cells are located in the intestinal lamina propria, such as T, B, natural killer (NK), NKT cells, macrophages (M ϕ), dendritic cells (DCs), mast cells, neutrophils, eosinophils, as well as stromal cells (such as fibroblasts). It is actually surprising that the large lymphoid system in the intestine coexists so peacefully with the external environment, a single epithelial layer away from the luminal microbial flora. However, under inflammatory conditions, a large number of activated immune cells infiltrate into the intestinal mucosa. These immune cells and some stromal cells not only express high levels of adhesion molecules and auxiliary signal molecules (such as CD54, CD62L), but also express high levels of inflammatory mediators and chemokine receptors (such as CCR5, CCR6, and CCR9) and integrin (such as integrin $\alpha 4\beta 7$). Moreover, fibroblasts and capillary endothelial cells in the intestinal mucosa also express high levels of chemokines, selectins (*e.g.*, selectin E) and intracellular adhesion molecule-1 (ICAM-1, or CD54), which further induce intermolecular interactions of leukocytes in the blood circulation to migrate into the intestinal mucosa, and promote local inflammatory response^[1-4].

Evidence has demonstrated that CD4⁺ T cells isolated from inflamed mucosa of CD patients, when stimulated *in vitro*, are able to produce large amounts of Th1/Th17-associated proinflammatory cytokines (*e.g.*, IFN- γ , TNF, and IL-17A), while in UC inflamed tissue CD4⁺ T cells and NK T cells secrete high levels of Th2-related cytokines (*e.g.*, IL-4 and IL-13) and Th17-associated proinflammatory cytokines (*e.g.*, IL-17A)^[1-4]. The unbalance of pro/antiinflammatory cytokines contributes to intestinal mucosal inflammation. Recent studies have found that some proinflammatory cytokines (*e.g.*, IL-12, IL-18, IL-21, and IL-23) are significantly increased in inflamed mucosa of CD patients^[5-7], and that some inhibitory cytokines (*e.g.*, TGF- β , IL-10, IL-25, IL-33, and IL-37) are significantly reduced. Moreover, loss of forkhead proteins (Foxp)3⁺ regulatory T cells (Treg) and FoxP3 IL-10⁺ CD4⁺ cells are also found in the inflamed mucosa of IBD patients, and these events result in not maintaining intestinal mucosal immune tolerance and further promoting local intestinal mucosal immune response, leading to

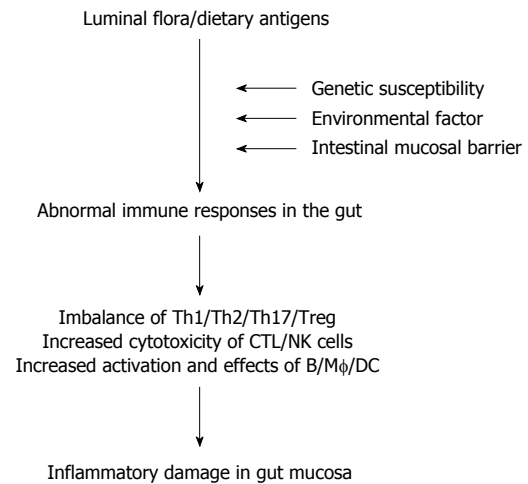


Figure 1 Pathogenesis of inflammatory bowel disease. DC: Dendritic cell; NK: Natural killer; Th: T helper; Treg: T regulator cell; CTL: Cytotoxic T lymphocyte; M ϕ : Macrophages.

the intestinal mucosal injury^[8].

PLEIOTROPIC ROLE OF IL-21 IN IMMUNE RESPONSE

IL-21 is a member of the IL-2 family of cytokines, expressed mainly by CD4⁺ T cells, including Th1, Th2, and Th17 cells (Figure 2)^[9,10]. IL-21 receptor (IL-21R) is structurally related to IL-2R and IL-15R, and is expressed in T, NK, B cells, and DCs^[9]. IL-21 exhibits a pleiotropic capacity to regulate T cell differentiation and function, enhances clonal expansion of antigen-activated naive CD4⁺ and CD8⁺ T cells, and induces the expression of genes encoding IL-12R, IL-18R, IFN- γ , IL-2R α , and the Th1-associated transcription factor T-bet in activated memory T cells^[11,12]. IL-21 is also associated with the Th2-mediated immune response and plays a role in inhibiting the differentiation of naive Th cells into IFN- γ -producing Th1 cells. In synergy with IL-15, Fc γ 3 ligand, and stem cell factor, IL-21 promotes human NK cell maturation and activation. It exerts further biological functions in B cells, regulates differentiation and antibody production, including production of all IgG isotypes, and synergizes with anti-CD40 mAb to stimulate B-cell activation, clonal expansion, and maturation (Figure 2)^[13-15].

In recent years, IL-21 has been found to be produced in excess in the intestine of IBD patients and may be involved in the pathogenesis of human IBD^[16-18]. When mucosal T cells from CD patients are activated *in vitro* with anti-CD3 in the presence of either a neutralizing anti-IL-21 antibody or an IL-21R-IgG fusion protein, the production of both IL-17A and IFN- γ is reduced. These results together with the demonstration that IL-21-deficient mice are resistant against Th1/Th17 cell-driven colitis support the key role of IL-21 in positively regulating Th1 and Th17 cell-associated inflammatory pathways. IL-21 exerts further biological functions that could contribute to its proinflammatory effect in the gut. For

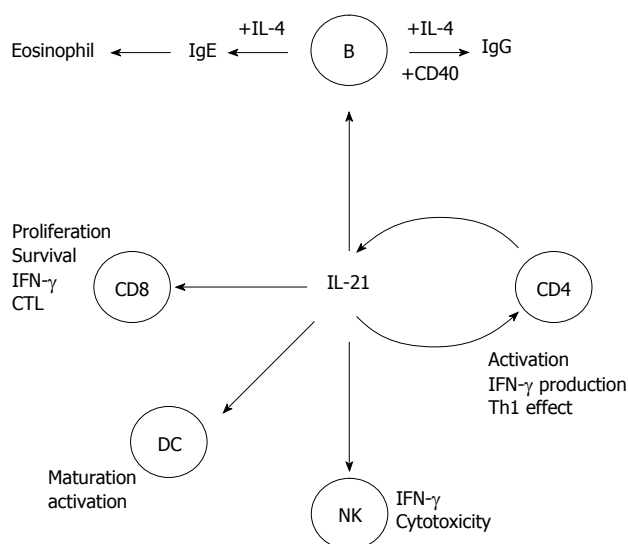


Figure 2 Pleiotropic role of interleukin-21 in immune responses. IFN: Interferon; IL: Interleukin; CD: Crohn's disease; DC: Dendritic cell; NK: Natural killer; CTL: Cytotoxic T lymphocyte.

example, IL-21 stimulates stromal cells to produce tissue-degrading proteases and enhances secretion of the T-cell chemoattractant macrophage inflammatory protein-3α by intestinal epithelial cells. IL-21 potentiates the expression of Th1-related transcription factors and IFN-γ in T and NK cells and the cytotoxic activity of NK cells. IL-21 also inhibits the peripheral differentiation of Tregs and makes CD4⁺ T cells resistant to Treg-mediated immune suppression. Therefore, through the multiple pathways IL-21 can damage the gut, and neutralizing IL-21 may have a therapeutic potential in the management of IBD^[13,18].

We have also investigated expression of IL-21R in inflamed mucosa of IBD patients and evaluated its role in the induction of NK cell cytotoxicity and activation as well as Th17 differentiation^[5]. The results have shown that IL-21R-positive cells are significantly increased in inflamed mucosa of IBD patients compared with healthy controls, and IL-21R is mainly expressed in freshly isolated peripheral blood (PB)- and lamina propria (LP)-CD4⁺, CD8⁺ T, B, and NK cells. When stimulated with immobilized human IgG and IL-21, PB-NK cells from IBD patients produce higher levels of IFN-γ and TNF than those from controls. IL-21-primed IBD NK cells show a more potent antitumor cytotoxicity to NK-sensitive K562 cells than controls. Moreover, PB-T and LP-T cells from IBD patients produce larger amounts of proinflammatory cytokines (*e.g.*, TNF and IFN-γ) than those from controls when stimulated with IL-21 and anti-CD3. Importantly, IL-21 facilitates IBD CD4⁺ T cells to differentiate into Th17 cells^[5]. In our further study, we have also evaluated the role of anti-TNF mAb (infliximab, IFX) in regulating IL-21 expression and Th17 cell infiltration in the intestinal mucosa of CD patients. Twenty-six CD patients were treated with IFX at weeks 0, 2 and 6. IL-21 and Th17 cells were found to be expressed highly

in inflamed mucosa of active CD patients compared with healthy controls. Ten weeks after IFX infusion, CD activity index, erythrocyte sedimentation rate, serum C-reactive protein (CRP) and intestinal mucosal healing were improved markedly in CD patients. Moreover, IL-21 expression and Th17 cell infiltration were also found to be significantly decreased compared with those before IFX therapy^[19]. These data indicate that IL-21 plays an important role in the pathogenesis of IBD.

PROINFLAMMATORY ROLE OF IL-23 IN THE PATHOGENESIS OF IBD

Recent advances have also indicated that IL-23, mainly produced by macrophages, is one of the critical cytokines in IBD and is essential for promoting chronic intestinal inflammation^[20,21]. IL-23 and IL-12 are members of a small family of proinflammatory heterodimer cytokines, sharing a common p40 subunit covalently linked to a p35 subunit to form IL-12 or to a p19 subunit to form IL-23. IL-12R is comprised of an IL-12Rβ1 and IL-12Rβ2 subunit, whereas the receptor for IL-23 consists of the IL-12Rβ1 subunit and a novel component termed IL-23R^[20], which is expressed predominantly on T, NK, and NKT cells and to a smaller extent, on monocytes, macrophages, and DCs. After binding to the IL-23R, IL-23 preferentially induces memory T cell activation. IL-23 exhibits some similar biological activities to IL-12, however, in comparison with IL-12 with profound induction of the Th1 immune response, as well as promotion of cytotoxic, antimicrobial, and anti-tumor responses, IL-23 is found to play a critical role in the maintenance of immune response by controlling T cell memory function and by influencing the proliferation and survival of IL-17-producing Th17 cells^[22,23]. Moreover, recent work has also shown that IL-23 could induce naive CD4⁺ T cells to secrete IL-22, indicating that IL-23 is also associated with the differentiation of naive CD4⁺ T cells^[24].

Previous studies with murine colitis models have demonstrated a requirement for IL-23 in the development of intestinal mucosal inflammation. IL-23p19 subunit knockout results in a decrease of proinflammatory cytokines (*e.g.*, TNF, IFN-γ, IL-6, and IL-17) and the presence of less intestinal mucosal inflammation^[25,26]. Moreover, *in vivo* blockade of IL-23 using anti-IL-23p19 mAb or its inhibitor STA-5326 could inhibit chronic intestinal inflammation in a colitis model and down-regulate a Th1-mediated immune response^[26,27]. Elevation of IL-23p19 transcript levels has been observed in inflamed mucosa of IBD patients, and its expression is correlated with the severity of endoscopic lesions^[28]. Recent work has demonstrated that myeloid DCs from mesenteric lymphoid nodes of CD patients, when stimulated with exogenous microbial antigens *in vitro*, produce higher levels of IL-23p19 than UC patients and healthy controls^[29].

In order to investigate the pathogenic role of IL-23 in the induction of mucosal inflammation in IBD, we have analyzed IL-23p19 expression in inflamed mucosa

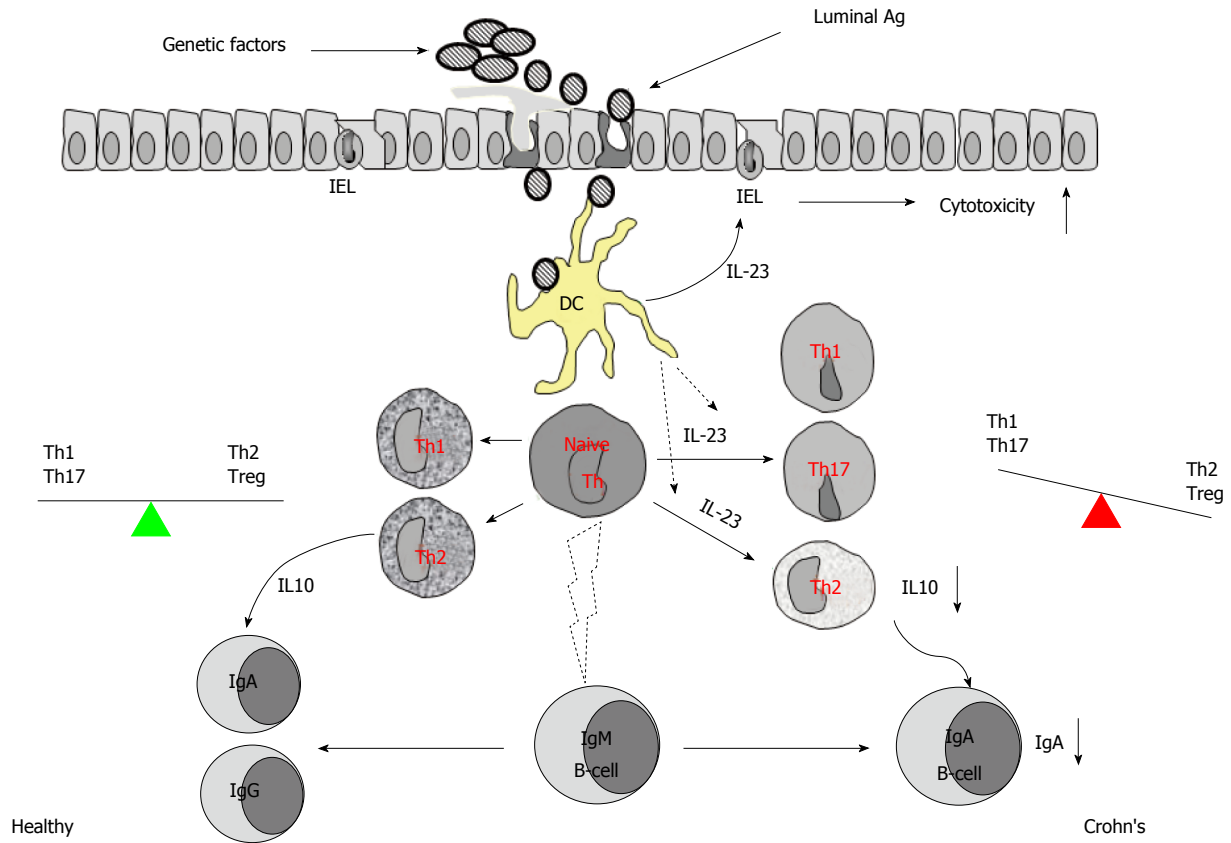


Figure 3 Overexpression of interleukin-23 in inflamed mucosa of patients with inflammatory bowel disease weakens the intestinal defensive barrier and disturbs the immune regulation in intestinal mucosa. IEL: Intestinal epithelial lymphocyte; IL: Interleukin; Th: T helper; Treg: T regulator cell; DC: Dendritic cell.

of IBD patients and its role in the induction of intestinal epithelial lymphocyte (IEL) and NK cell activation as well as Th17 cell differentiation. Expression of IL-23p19 has been observed to be increased significantly in inflamed mucosa of CD patients compared with that in UC patients and healthy controls. IL-23R cells are increased significantly in PB- and LP-CD4⁺ and -CD8⁺ T and NK cells. IL-23 could markedly promote IBD IEL and NK cell activation and cytotoxicity and triggered IBD PB- and LP-T cells to secrete significantly higher levels of IFN- γ , TNF, IL-2, and IL-17A compared with healthy controls. IL-23 promotes IBD PB- or LP-CD4⁺ T cells to differentiate into Th17 cells. These data indicate that IL-23 plays an important role in the induction of IEL, NK, and T cell activation, proinflammatory cytokine secretion, and Th17 cell differentiation^[6]. In two IBD models there is excessive accumulation of short-lived neutrophils and inflammatory monocytes in the intestine. IL-23-driven colitogenic T cell program has been found to regulate upstream hematopoietic stem and progenitor cells (HSPC)^[30]. Targeted therapy directed against IL-23 may have a therapeutic role in treatment of IBD.

Additionally, we have also elucidated the further role of IL-23 in the suppression of IL-10 in the IBD intestinal mucosa^[7]. IL-10 is an important cytokine in the induction of Th2 response that plays a crucial role in adaptive immunity *via* the induction of specific antibody-

ies to eliminate the reinvasion of microbes and the absorption of microbial products. IL-10 is also one of the most effective immune regulatory cytokines contributing to maintaining the homeostasis of the body^[31,32]. Previous studies indicate that the production of IL-10 in the intestine of IBD patients is suppressed, but the underlying mechanism has not fully understood yet. Therefore, we examined the expression of IL-10, IL-23, and IgA in the surgically removed colon specimens and found that the levels of IgA and IL-10 were significantly lower, and both negatively correlated with IL-23 expression and the infiltration of inflammatory cells in the IBD mucosa. The production of IL-10 by lamina propria mononuclear cells was lower in the IBD group than the control, and these levels could be enhanced by blocking IL-23. The gene transcription of IL-10 was significantly suppressed in CD4⁺ T cells of IBD mucosa, and this phenomenon could be replicated *in vitro* by adding IL-23 in the culture of polarized Th2 cells^[7]. Thus we conclude that overexpression of IL-23 in the intestinal mucosa weakens the defensive barrier in the gut and disturbs the local immune regulation (IL-10 and Treg) (Figure 3).

POTENTIAL ROLE OF IL-25 IN THE DEVELOPMENT OF IBD

IL-25 (also known as IL-17E) is a distinct member of the

IL-17 family of cytokines, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F. IL-25 shares the receptor IL-17 receptor homolog 1 (IL-17Rh1) (also named the IL-17RB) with IL-17B, although it binds with a much higher affinity. IL-25R is a 56-kDa single transmembrane protein and is expressed in Th2 central memory cells, eosinophils, monocytes, airways smooth muscle cells, fibroblasts, and endothelial cells. Evidence has shown that IL-25 is also involved in the immune responses in gut mucosa^[33]. Previous work has demonstrated that IL-25 is constitutively expressed by intestinal mucosal T cells of mouse strains (*e.g.*, BALB/c, C57BL/6 mice) that are resistant to helminth *Trichuris muris* infection, whereas IL-25-deficient mice on a genetically resistant background fail to develop a Th2-mediated immune response or eradicate *Trichuris* infection but develop severe infection-induced intestinal inflammation. Moreover, the immunopathology in *Trichuris*-induced IL-25-deficient mice is also associated with increased expression of IFN- γ and IL-17A in the mesenteric lymph nodes and cecum^[33]. Administration of IL-25 could prevent intestinal mucosal inflammation in experimental colitis induced with peptidoglycan, 2,4,6-trinitrobenzenesulphonic acid, or oxazolone in mice. These data indicate that IL-25, which promotes the differentiation and activation of Th2 cells in gut mucosa, plays a critical role in the attenuation of destructive intestinal inflammation^[33,34].

IL-25 has been also found to be decreased in the inflamed mucosa of IBD patients and could decrease the synthesis of IL-12 and IL-23 in the CD14⁺M ϕ from the inflamed mucosa of patients with CD *in vitro*^[35]. Therefore, IL-25 may be a negative regulator of inflammatory responses in the intestinal mucosa. However, the exact role of IL-25 in the development of IBD remains to be elucidated. Recently, we have also studied the role of IL-25 in the pathogenesis of IBD^[36]. The results have demonstrated that IL-25 is significantly decreased in the sera and inflamed mucosa of patients with active IBD compared with controls. The levels of IL-25 in inflamed mucosa and sera are inversely correlated with endoscopic disease activities and CRP, respectively, in IBD. IL-25 could markedly inhibit IBD CD4⁺ T cells to produce TNF, IFN- γ , and IL-17A but promote IL-10 secretion. IL-25 could suppress the differentiation of IBD CD4⁺ T cells into Th1 and Th17 cells but did not interfere with Th2 cell differentiation. Importantly, blockade of IL-10 secretion by IBD CD4⁺ T cells markedly attenuates the inhibitory role of IL-25 in modulating both Th1 and Th17 immune responses (Figure 4). Our study provides evidence that IL-25 is a critical anti-inflammatory cytokine in the pathogenesis of IBD and may be considered as a potential therapeutic agent for human IBD^[36].

POTENTIAL ROLE OF THE TH17/IL-17 AXIS IN THE PATHOGENESIS OF IBD

IL-17 has pleiotropic activities, functions through the adaptive and innate immune system to promote im-

mune response, and plays an important role in immune responses. The identification of the IL-17 family of cytokines as well as the IL-23-mediated expansion of IL-17-producing T cells has uncovered a new subset of Th cells, designated as Th17 cells. Th17 cells originate from naive CD4⁺ T cells in the presence of TGF- β and IL-6. Th17 cell differentiation does not require IL-17. The amplification and stabilization of Th17 are provided by IL-21 and IL-23. At the same time, the ROR γ t is identified as the master key regulator and transcription factor of Th17 cell differentiation^[37,38]. The IL-17 cytokine family includes six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (or IL-25) and IL-17F, and act *in vitro* and *in vivo* as potent proinflammatory cytokines^[38]. IL-17A can induce the expression of proinflammatory cytokines (such as IL-6 and TNF), chemokines (such as KC, MCP-1 and MIP-2) and matrix metalloproteases, which mediate tissue infiltration and tissue destruction. It is also involved in the proliferation, maturation and chemotaxis of neutrophils^[38].

Evidence has shown that high numbers of Th17 cells are present in the colonic LP of the ileum and colon in conventionally raised mice, and that these cells are highly infiltrated in inflamed areas of colitic mice^[39,40]. Further analysis confirms that commensal gut flora contributes to the expansion of these CD4⁺ Th17 cells, leading to intestinal mucosal inflammation. In terms of mucosal immunity, the IL-23/IL-17 axis has been observed to play an important role in normal intestinal homeostasis.

To date, IL-17 and other Th17-associated cytokines (*e.g.*, IL-22 and IL-23) have been found to have protective or pathogenic effects dependent on other effective factors in local tissue. Recent work^[41] has also demonstrated that most of the transcripts for Th17-related cytokines are increased in IBD patients compared to normal controls, but more abundant in UC than in CD. In contrast, upregulation of IFN- γ mRNA is marked in CD LP CD4⁺ T cells. Up-regulation of IL-23p19 mRNA is detected in colonic mucosa from both UC and CD patients. The significance of Th17 immunity in UC is further supported by the finding that recombinant IL-23 actually enhances IL-17A production by LP CD4⁺ T cells in UC, but has a lesser effect on LP CD4⁺ T cells in CD^[42]. IL-17A is protective against dextran sodium sulfate (DSS)-induced colitis and colitis in the T cell transfer model, in which T cells are injected into lymphopenic mice. However, mice deficient in IL-17F are resistant to DSS-induced colitis. These data suggest that IL-17F, not IL-17A, is pathogenic in the gut. IL-17 may synergize with other inflammatory mediators in the gut. Recent studies have highlighted further potential heterogeneity within Th17 cell populations by demonstrating that some may even secrete IL-10^[43], a factor known to inhibit intestinal inflammation. Thus, it is possible that the actions of Th17 cells may differ dependently on other factors that may be present in the local environment. In the normal intestine, the primary function of Th17 cells may be like sentinels which contribute to maintaining epithelial barrier function, whereas

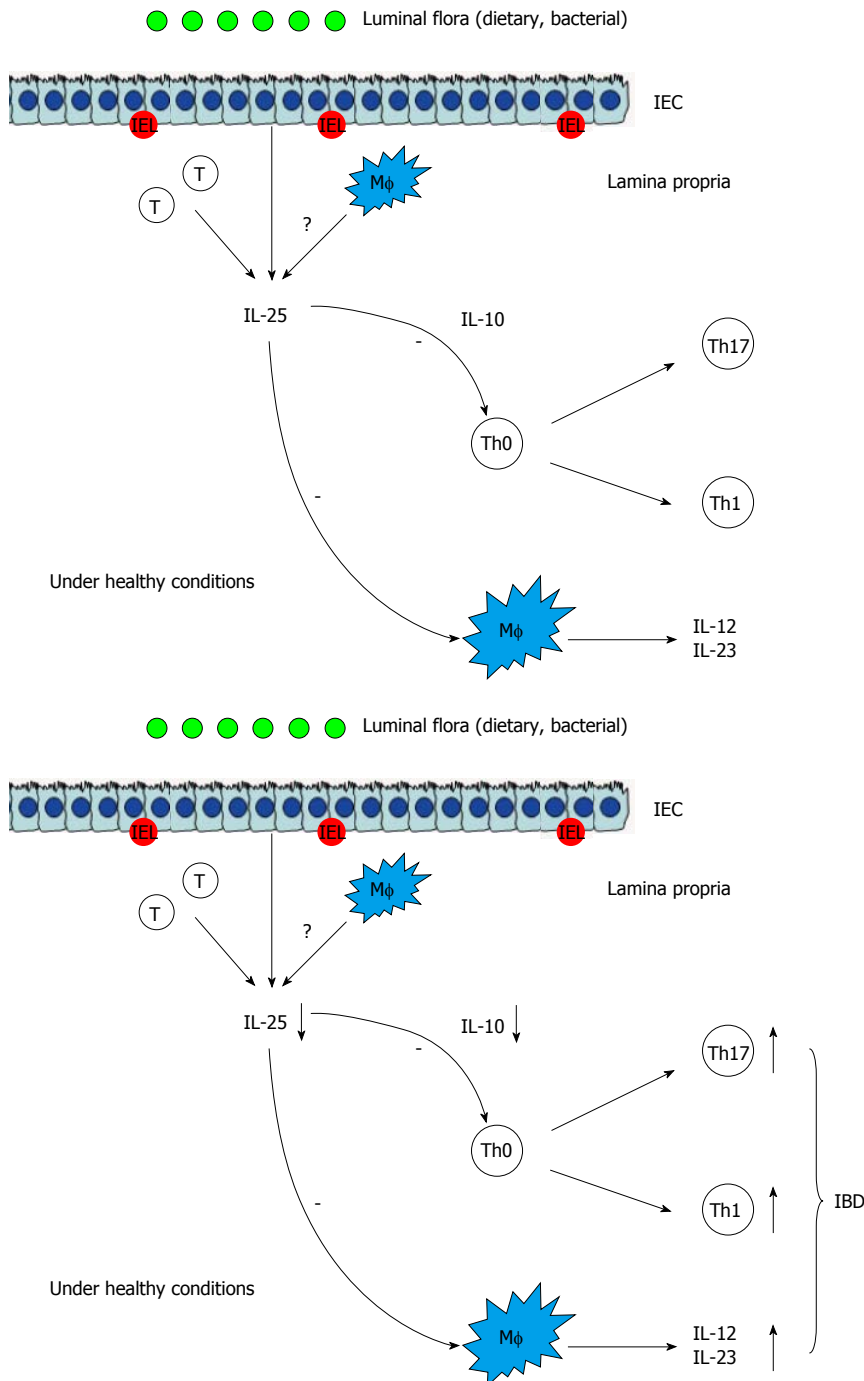


Figure 4 Inhibitory role of interleukin-25 in intestinal mucosa. IEL: Intestinal epithelial lymphocyte; IL: Interleukin; Th: T helper; Treg: T regulator cell; CTL: Cytotoxic T lymphocyte; Mφ: Macrophages; IEC: Intestinal epithelial cells; IBD: Inflammatory bowel disease.

in sites of chronic intestinal inflammation, high levels of IL-23 may activate their full pathogenic and antibacterial functions. Recently, Secukinumab (an IL-17A antibody), Brodalumab (an IL-17 receptor antibody) and two small-molecule drugs (Vidofludimus and Tofacitinib) are used in clinical trials for IBD patients, which inhibit IL-17 as part of their overall pharmacological profiles^[44].

IL-27 AND IL-35: NEWER MEMBERS OF THE IL-12 FAMILY

The IL-12 family is made up of secreted heterodimers

with some overlapping usage between family members. IL-12 (p35/p40) and IL-23 (p19/p40) are the best-known members of the IL-12 family. Other members include IL-27 (EBi3/p28) and IL-35 (EBi3/p35). Like the IL-17 family, however, a critical issue is whether these molecules are pathogenic or protective in the gut. IL-27, produced mostly by myeloid cells, is present at increased concentrations in IBD mucosa. If T cells are taken from mice deficient in the IL-27R and injected into lymphopenic mice, they induce significantly less colitis than wild-type T cells, clearly suggesting that IL-27 is pathogenic in this model. IL-27R-null mice are also less susceptible to DSS-induced

colitis, again suggesting that IL-27 is a proinflammatory mediator. In marked contrast, however, treating mice with established TNBS-induced colitis with IL-27 reduces disease and cytokine production. In humans, IL-27 has also been observed to reduce proinflammatory cytokine production in M ϕ activated with TNF^[45].

MICRORNA AND THE INTESTINAL IMMUNE HOMEOSTASIS

MicroRNA (miR) is an emerging group of short, non-coding RNAs that play an important role in regulating expression of classical genes at the post-transcriptional level. miR regulates cell proliferation, apoptosis, growth, cell differentiation, metabolism and other processes. Recently, miR in intestinal epithelial cells has been found to regulate intestinal mucosal barrier function through its important effect on intestinal epithelial cell proliferation and differentiation. Moreover, differential expression of miR in immune cells within the intestinal mucosa affects the intestinal immune homeostasis^[46,47].

In recent years, evidence has suggested that intestinal epithelial miR expression is closely related with the incidence and development of IBD^[46]. The expression of miR-192, miR-375 and miR-422b is significantly decreased in inflamed mucosa of patients with active UC, while miR-16, miR-21 and let-7 expression is significantly increased. miR-19b and miR-629 are significantly decreased in patients with active CD inflammation within the intestinal mucosa, while miR-23b, miR-106 and miR-191 are significantly increased. The abnormal miR expression will affect translation process of its corresponding target gene mRNAs, regulate gene expression and thus participate in inflammatory injury of intestinal mucosa in IBD. A recent study by Coskun *et al*^[48] provides the first evidence that miR-20b, miR-98, miR-125b-1*, and let-7e* are deregulated in patients with UC.

We have also investigated the expressions of miR10a in IBD and found that the expression of miR10a is decreased in inflamed intestinal mucosa of IBD patients. Furthermore, we have also found that TNF inhibits miR10a expression, while blockade TNF with anti-TNF mAb markedly enhances miR10a expression in the intestinal mucosa (unpublished data). Our findings, together with previous data showing that miR10a could block intestinal inflammation in mice and reduce the differentiation Th1 and Th17^[49], further prove that miR10a is involved in intestinal mucosal inflammatory response, and that targeted therapy may be beneficial for human IBD.

ROLE OF REGULATORY B CELLS

B cells are a source of inhibitory cytokines such as IL-10 and TGF- β . Depending on the signals B cells receive, pro- or antiinflammatory cytokines can be produced, and the shift towards an inflammatory or a protective/suppressive response will be induced. Specific B cell subset found to affect autoreactive responses and suggested to

have a regulatory role in autoimmune diseases is B-regulatory cells (Bregs). CD19⁺CD25⁺ B cells were the first subset of human B cells previously suggested to have a regulatory role. CD19⁺CD25⁺ B cells contribute up to 30% of all peripheral blood B cells in mice and can effectively present peptides to helper T cells. In humans, only B cells expressing high levels of CD25 (BCD25⁺) seem capable of acting as Bregs with abundant TGF- β production. However, it remains unknown how to identify Bregs with membrane markers or transcription factors. Several signals like B cell receptor (BCR), CD40 and/or Toll-like receptor (TLRs) may include. These different activation signals (alone or combined) were shown by many studies to increase regulatory functions of Bregs^[50].

Bacterial molecules have been used to stimulate Bregs. In mice, splenic B cells stimulated *ex vivo* by bacteria acquire the CD5⁺ CD1d⁺ phenotype, which is characterized by high level IL-10 expression and being capable of markedly suppressing the activity of experimental IBD. The protective subset (contributing 1% to 2% of all splenic B cells) is composed of CD5⁺CD1d(high) B cells activated *via* the TLR2/4 pathway by bacterial antigens in the gut flora. Investigations into the influence of the gut microbiota on the balance between effector B cells and Bregs may open up new therapeutic possibilities in IBD^[51,52].

ROLE AND FUNCTIONAL ALTERATIONS OF THE INNATE IMMUNITY

Innate immunity prevents pathogens from entering and spreading within the body. The intestinal innate immune system involves three lines of defense: the mucus layer, epithelium, and lamina propria. Mucus is the first line of intestinal defense, and the major constitutive proteins are mucins (MUC), with diverse isotypes in different portions of the gastrointestinal tract. CD patients show decreased MUC1 and MUC4 levels in the ileum, while MUC2, MUC5AC, MUC5B, MUC6, and MUC7 are undetectable in lesions. UC patients also show decreased MUC2 expression. As the second line of defense, the intestinal epithelium is composed of a monolayer of fast replicating polarized cells: enterocytes, goblet cells, and enteroendocrine cells. All are bound together through tight junctions that separate the body from intestinal lumen components. The membrane TLRs and cytosolic nucleotide oligomerization domain receptor (NOD) are the most important among intestinal pathogen recognition receptors. NOD2 has also been described as a negative regulator of TLR2-mediated IL-12 secretion. Some NOD2 mutations have been described in CD patients, which are associated with decreased defensin secretion by ileal mucosa Paneth cells.

Lamina propria as a third line of intestinal defense contains innate and immune cells. In IBD patients, DCs promote a robust recognition of bacterial products that might cause an immune response to commensal bacteria, provoking a loss of intestinal tolerance. Intestinal macro-

phages from IBD patients have lost the ability to maintain tolerance, mainly through increased surface CD14 content and NF- κ B transcription pathway activity, which might induce increased peripheral macrophage recruitment. Increased cytotoxic activity and elevated NK cell counts are also found in IBD patients. Crucial molecules in NK cells, such as IL-15, IL-21 and IL-23, and their cognate receptors, are elevated in the intestinal mucosa of UC patients. Moreover, in IBD the IEL activation increases, resulting in elevated production of IFN- γ , TNF and IL-2, which is associated with increased IL-23^[53,54].

Deregulated mucin expression in IBD patients might be due to the cytokine imbalance that characterizes these diseases. These molecules stimulate various transcription factor pathways, such as JAK/STAT and NF- κ B, and induce mucin secretion. The combination of TNF and IFN- γ could also decrease claudin-3, claudin-5 and claudin-7 expression, with a marked increase in paracellular permeability in rat colon. Moreover, TNF, IL-6 and IFN- γ increase apoptosis and monolayer permeability in HT-29/B6 cells. These cytokines also inhibit the wound-healing in HT-29/B6 cells. Increased apoptosis and delayed woundhealing of epithelial cells would augment monolayer permeability, and damage the epithelial barrier function^[54,55].

CONCLUSION

The exact etiology of IBD is still not completely understood, and increasing data have demonstrated that these conditions occur through an inappropriate immune response to a subset of commensal enteric bacteria in a genetically susceptible host, with disease initiated by environmental triggers. Dysfunction of the mucosal immune system evokes intestinal inflammation through the activation of both innate and acquired immunity in the gut. Among these, T cell activation and Teff/Treg imbalance play an important role in the process of inflammation. Understanding of immunopathogenesis of IBD will help us to find new ideas for diagnosis and treatment.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Restoring the gut microbiome for the treatment of inflammatory bowel diseases

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Core tip: Advances into the understanding of the pathogenesis of inflammatory bowel diseases (IBD) have highlighted the importance of a dysbiosis in the intestinal microbiome. A perturbed microbiota with loss of colonization resistance is a main driver of *Clostridium difficile* infection and exciting new data exists that microbial restoration through the use of fecal microbiota transplantation (FMT) is highly successful. Therefore, it is logical to conclude that FMT will have therapeutic efficacy in IBD. Preliminary studies that have evaluated FMT for IBD are reviewed with an emphasis on subpopulations that may benefit the most. The limitations and unknowns for this novel therapy are also discussed.

Abstract

Fecal microbiota transplantation (FMT) is considered to be a highly successful therapy for recurrent and refractory *Clostridium difficile* infection (CDI) based on recent clinical trials. The pathogenesis of inflammatory bowel diseases (IBD) is thought to be due in part to perturbations in the gut microflora that disrupt homeostasis. FMT restores essential components of the microflora which could reverse the inflammatory processes observed in IBD. Case reports and series for the treatment of IBD by FMT have shown promise with regards to treatment success and safety despite the limitations of the reporting. Future studies will determine the optimal delivery and preparation of stool as well as the conditions under which the recipient will derive maximal benefit. The long term consequences of FMT with regards to infection, cancer, auto-immune, and metabolic diseases are not known and will require continued regulation and study. Despite these limitations, FMT may be beneficial for the treatment of ulcerative colitis and Crohn's disease, particularly those with concurrent CDI or with pouchitis.

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease, the two main classifications of inflammatory bowel diseases (IBD) are characterized by chronic intestinal inflammation resulting in recurrent episodes of disease exacerbations with associated abdominal pain, diarrhea, weight loss and rectal bleeding. IBD remains poorly understood and medical therapies continue to be inadequate. The current mainstays of conventional therapy for these diseases include 5-aminosalicylates (5-ASAs), corticosteroids, thiopurines, and anti-tumour necrosis factor agents. However, despite continued advances in therapy, a significant number of

patients remain refractory to standard therapies. Overall, 20%-30% of patients with UC will require a colectomy. At least 50% of patients with Crohn's disease will require surgical treatment in the first 10 years of disease and 70%-80% will require surgery within their lifetime^[1].

The etiology of IBD is complex and several factors are believed to play a role in its development and progression. The host genotype is important and twin studies have shown concordance rates of 16% for UC and 35% for Crohn's disease^[2]. However, these numbers also indicate that non-genetic factors play a substantial role in the development of IBD^[3]. The most important of these is likely the intestinal microbiota (reviewed extensively in this issue of *WJG*). Humans have evolved with the microbes in the intestine which are known to provide critical functions to the host such as metabolism, digestion, development and maintenance of the immune system, and mucosal barrier function. The microbes exist in the various niches to carry out their function and are relatively stable over time^[4]. In disease states such as IBD, the microbial balance that favored homeostasis is perturbed and studies that have analyzed the composition of the gut microbiome in IBD have found a loss in the richness and diversity of the bacterial components including under representation of the anti-inflammatory phyla *Bacteroides* and *Firmicutes* and a relative plume of pro-inflammatory *Proteobacteria*^[5-7]. This shift in the composition of the microbiota ("dysbiosis") may favor the appearance of distinct pathogens that perpetuate the inflammatory response. In this regard, several studies have revealed an increase in adherent/invasive *E. coli* in the terminal ileum of patients with Crohn's disease and *Mycobacterium avium paratuberculosis* has been casually linked to Crohn's pathogenesis although a direct link has not been proven^[8]. Opportunistic microbes such as *C. difficile* may also be able to establish pathogenicity in niches that may be present in the colons of IBD patients. Whether the dysbiosis directly leads to inflammation or is a consequence of an inflammatory environment is yet to be determined. Nonetheless, antibiotics and fecal diversion have been successful in treating various forms of IBD^[9], and it is possible that restoring a healthy microbiota through Fecal microbiota transplantation (FMT) may prove to be more effective^[2].

FMT has been suggested as a therapy for IBD, given the observed intestinal dysbiosis^[10]. FMT has also been termed "fecal bacteriotherapy", "human probiotic infusion", "stool transplant," "intestinal microbiome restoration" and "fecal transfer" in the literature. FMT involves collecting stool from a healthy pre-screened donor and delivering a prepared slurry into the gastrointestinal tract of the individual with disease via nasogastric tube, EGD, colonoscopy, or enema^[10]. Multiple studies have investigated the role of FMT for the treatment of colitis and diarrhea caused by the opportunistic pathogen *C. difficile*. The accumulated data suggests that FMT is a safe and highly effective therapy for *C. difficile* infections (CDIs) refractory

to standard medical treatment with antibiotics^[11-22]. In this review, we will discuss the literature on the use of FMT for the treatment of IBD with a focus on special populations of patients with IBD who are predicted to respond to this treatment. We also discuss the limitations of FMT and remaining questions for this exciting novel therapy.

PUBLISHED EXPERIENCE WITH FMT AS A THERAPY FOR IBD

There are currently no published clinical trials on FMTs in either UC or Crohn's disease. The literature consists of various case reports and case series, mainly in UC. The first report of FMT for UC was presented by Bennet and Brinkman in 1989^[23]. Bennet, who had UC, self treated with fecal retention enemas. Six months after his experimentation, he remained symptom-free and off of medications. This report was followed by a case series, by Borody and colleagues in the same year, of 55 patients with a mixture of gastrointestinal disorders including UC, Crohn's disease (only one patient), and irritable bowel syndrome who were treated with FMT by retention enemas^[24]. They reported that 20 of the 55 patients were "cured" after one FMT and 9 had significant symptom reduction. This study was very limited and did not provide details as to how clinical outcomes were measured and which patient groups may have derived the greatest treatment benefit. Furthermore, details were not provided as to the frequency and duration of treatment or the length of follow up. The one patient with Crohn's disease was reportedly symptom free after four months after suffering from steroid refractory disease^[24]. In a later review, Borody reported that this Crohn's patient had relapsed at 18 mo^[25]. Borody followed up this report up with another case series highlighting 6 patients with UC^[26]. Each of the 6 had at least five years of disease and had either failed what was described as maximal medical therapy (steroids, 5-ASAs and mercaptopurine) or quickly relapsed upon withdrawal of medications. Each patient was confirmed to have active inflammation on colonoscopy and was negative for CDI. Prior to the treatment, each patient received antibiotics for 7-10 d in order to suppress the *Clostridia* (vancomycin 500 milligrams (mg) twice daily, metronidazole 400 mg twice daily, and rifampicin 150 mg twice daily). Each patient also underwent a one time 3 L lavage with an oral polyethylene glycol solution. These patients provided their own donors and received daily fecal retention enemas for five days. They were encouraged to retain the enemas for as long as possible (6-8 h). Each of these patients was in complete remission four months after treatment and remained off of IBD medications. In several of the patients, follow up colonoscopy revealed no active inflammation. The follow up time was variable but remission was reportedly sustained over many years (1-13)^[26]. A recent systematic review on the topic found nine articles and 26 patients (18 UC, 6 CD, 2 indeterminate) who had received FMT for management of IBD, several of which are included

in the series described above^[27]. Of these 26 patients, results were reported in 17. After FMT, 13/17 patients were able to cease all IBD medications within 6 wk and all had symptom reduction or resolution at 4 mo^[28]. It is important to note that these cases varied significantly in the route of administration, preparation of stool, and screening protocols.

Angelberger *et al*^[29] characterized the bacteria communities present both pre and post FMT in 5 patients with moderate or severe UC. They found that none of the 5 patients achieved remission by week 12 and response was only noted in one patient. In two of the patients, further deterioration of their UC was noted at 4 wk post FMT. Upon analysis of the microbial compositions, they found that the UC patients pre FMT displayed a low phylotype richness and an overrepresentation of *Enterococcaceae* and *Enterobacteriaceae* and an underrepresentation of *Lachnospiraceae*, *Ruminococcaceae* and *Bacteroidaceae* when compared to healthy donors^[29]. They found that post FMT the microbiota of the patients became similar to that of the donor, however the duration of that change was patient dependent. The one patient with a clinical response maintained a similar microflora to the donor extending to 12 wk post FMT. However, the 2 patients who experienced disease deterioration showed increased microbiota dissimilarity by 4 wk post FMT. This small study raises several important questions such as what IBD phenotypes may respond best to FMT and how many infusions are necessary to establish a healthy microbiota and a sustained clinical response. Future studies for the treatment of IBD should carefully consider whether disease severity at the time of FMT affects treatment outcome, and at what point in the IBD disease process FMT may be optimal.

FMT FOR CDI IN PATIENTS WITH IBD

The incidence of CDI continues to rise^[30]. First line treatment for CDI consists of antibiotic therapy, however recurrence rates have been reported between 15%-35%^[30]. FMTs are best studied in CDI infections refractory to standard treatment. Current literature consists of multiple case series, systematic reviews and a recent randomized controlled trial^[31]. Impressively, cure rates have been reported between 81% and 100%^[30]. CDI infections are more common in patients with IBD, with a higher prevalence among patients with UC (3.7%) and Crohn's disease (1.1%) compared with the background general population (0.45%)^[32]. While IBD itself is thought to be an independent risk factor for CDI, the increased prevalence has also been linked to immunosuppressive medications, increased antibiotic use and multiple surgeries and hospitalizations^[33]. CDI may have adverse effects on the underlying IBD and so effective therapy to eradicate the organism is necessary to promote disease remission^[33]. A recent systematic review identified eight articles that reported on 15 patients (9 UC, 6 Crohn's disease) who underwent FMT for recur-

rent or refractory CDI, however outcomes were only reported in 12 of these patients^[27]. All patients had resolution of *C. difficile* as measured by stool specific testing. Several patients were noted to have fever and abdominal pain post FMT in this cohort but no major adverse events were reported. We will await the results of future trials to verify that FMT is a safe and effective therapy for IBD patients with *C. difficile*.

UC PATIENTS WITH ILEAL POUCHES

Up to 20% of people with UC undergo an ileal pouch anal anastomosis (IPAA)^[34]. Over 60% of individuals undergoing an IPAA for UC have at least one episode of pouchitis^[35]. This complication is uncommon in those undergoing IPAA for non-UC related reasons, such as familial adenomatous polyposis suggesting that genetic and environmental factors such as the composition of microbiota play a role in the pathogenesis of pouchitis^[36]. Its development in relationship to microbiota likely has two sides: a dysbiosis which reflects changes in bacterial composition possibly at the core of the pathogenesis of UC as well as the emergence of pathogenic bacteria such as *C. difficile*^[37]. Pouchitis often responds to a course of antibiotics but may recur and require multiple courses of the same antibiotic or a switch to a different antibiotic^[34]. Various types of probiotic preparations have been demonstrated to maintain remission in pouchitis when used daily^[38,39]. Unfortunately, pouchitis becomes a chronic, refractory condition in 5%-30% of patients undergoing IPAA for UC^[40] and more effective therapy is needed. Because dysbiosis likely propagates pouchitis in IBD and bacterial manipulation with antibiotics and probiotics has proven to be successful, it stands to reason that FMT will prove to be a successful treatment for many pouchitis patients. There is currently no published literature or ongoing trials exploring this possibility.

LIMITATIONS AND UNANSWERED QUESTIONS FOR FMT

Screening

The process of screening the donor stool and what tests should be ordered prior to FMT continues to evolve. Ideally, experts from gastroenterology and infectious disease can form a consensus regarding the appropriate screening of donor stool. In our practice, we ask the donor initially about high risk sexual behaviors, whether they have been diagnosed with any gastrointestinal diseases such as IBD, colon polyps, or irritable bowel syndrome, and whether or not they have taken antibiotics within the previous 3 mo. We then screen both the donor and recipient's blood for Hepatitis A (IgG and IgM), Hepatitis B (HBsAg/Ab and HBe), Hepatitis C (Ab), HIV-1/2 (Ab and viral load), and Syphilis (TP-IgG). The donor's stool is screened for *C. difficile* (by culture), routine stool bacterial culture, Giardia antigen,

Cryptosporidium antigen, and test for ova and parasites. More extensive screening protocols have been used in other studies that include additionally screening the donor for strongyloides, CMV, HTLV 1 and 2, EBV and Entamoeba histolytica^[31]. Given regional and geographic differences, we recommend consulting with an infectious disease specialist and infection control in order to determine the appropriate screening tests for an individual practice setting.

Whether or not the efficacy of FMT is improved with a related donor *vs* unrelated donor is not clear at this time. One recent systematic review suggested that stool from a related donor resulted in a higher resolution rate (90.5%) for CDI than an unrelated donor (84%)^[30]. However, other studies where universal donor pools were used have yielded similar overall results^[30]. Identification of individual bacterial components within the donor microbiota which could potentially influence efficacy are being investigated^[41].

Patient preparation

Most published reviews have recommended large-volume bowel lavage before the procedure, regardless of upper or lower tract administration in order to mechanically reduce *Clostridial* organisms that are still present^[30]. This concept has never been tested formally. For recurrent *C. difficile*, reports generally have recommended discontinuing antibiotics 1-3 d prior to the FMT^[13,30], however this has also not been compared to continuing antibiotics up until the day of the procedure. At our Institution, we have patients discontinue antibiotics the night before the FMT.

Stool delivery

Another issue that will need clarification through future study is the mode by which the stool is delivered to the bowel. Although the efficacy of FMT has been shown to be similar when delivered by endoscopy, nasogastric tube, enema or colonoscopy, it is possible that one method may be superior to another in IBD. The type and location of IBD may drive this decision. Upper gastrointestinal delivery of stool may be more efficacious for patients with small bowel Crohn's disease *vs* delivery by colonoscopy for patients with colonic disease. Lastly, tolerability and relative safety of each procedure will have to be considered when deciding between upper gastrointestinal delivery *vs* lower. In this regard, belching, nausea and abdominal cramps have been reported with upper gastrointestinal administration on the day of the procedure in 8/16 patients, however these symptoms resolved upon follow up^[31]. Although no major adverse events have been reported with any intestinal administration of stool, the safety of the proposed procedure should be considered at the time of treatment. Although colonoscopy is generally considered to be a safe treatment in the setting of active IBD, the perforation rate may be increased and other modes of stool delivery

should be considered in patients with moderate or severe inflammation or stricturing disease. Lastly, it is possible that FMT delivered by retention enema is effective in a subset of IBD patients and would obviate the need for an endoscopic procedure and hospital visit.

Processing and storage

Another unanswered question is whether donor stool may be frozen and then thawed prior to FMT. This has obvious practical implications but whether or not the key components of the stool will be adequately preserved is not known. The University of Minnesota reported similar *C. difficile* cure rates among patients who received fresh *vs* frozen (minus 80 degrees Celsius from 1-8 wk) specimens ($n = 33$)^[42]. In this series, 10 patients had underlying IBD. Interestingly, only 4 patients required a second FMT for recurrent symptoms and 3 of them had underlying IBD^[42]. Stool frozen at -80 °C therefore may be equally effective for FMT as fresh stool, however the viability of organisms after exposure to atmospheric oxygen may be an important consideration. Facultative anaerobes in stool may be inactivated by oxygen and thus transplants under anaerobic conditions may be more efficacious^[4].

It is conceivable that oral preparations that mimic human stool may be manufactured in the near future. Although probiotics have yielded modest treatment effects in certain populations of IBD^[43-46], it is likely that the various probiotics lacked critical organisms and possibly other factors that help successfully restore the gut microbiota back to health.

Long term complications

Whether or not FMT may exacerbate underlying bowel disease in some patients may be an important question. A case of a UC flare after FMT for CDI was recently reported^[47]. The patient had quiescent disease for twenty years and was not on immunosuppressive medications. He developed symptoms nine days after the FMT. *C. difficile* testing was negative and sigmoidoscopy revealed the appearance of inflammation and ulceration that was not present on the FMT colonoscopy.

There is a theoretical concern for the transmission of infections that may have escaped the screening process^[48]. A recent case series reports two patients who experienced gastroenteritis only 2 d and 2 wk after FMT respectively. Both patients were found to be *C. difficile* negative by PCR but norovirus positive. The donor stool however was negative for norovirus and the authors concluded that there was not direct transmission from donor to patient.

Whether or not FMT may influence non-gastrointestinal diseases in the long term such as metabolic disease, obesity, and cardiovascular disease is not known at this time^[49-53]. This may be the reason why several regulatory agencies such as the United States Food and Drug Administration have asked for more research on FMT

before this can be recommended as a first line treatment.

Regulation

While initial reports of FMTs for IBD are promising, several unresolved issues remain. Treatment of IBD with FMTs may be considered investigational and so many health care providers may not cover the cost of the procedure (colonoscopy and stool preparation). This is the case in the United States where the Food and Drug Administration has required that providers who would like to perform FMT must file an “Investigational New Drug” application. Many patients may end up having to pay the hospital charge for this treatment out of their own funds. This burden may be greater when it is possible that patients with IBD may require several treatments. Nonetheless, it is conceivable that long term costs may be reduced if FMT leads to treatment success and the patient is able to avoid expensive medical therapies, hospitalizations, or surgeries.

CONCLUSION

FMT is now considered to be a highly successful therapy for recurrent and refractory CDI based on recent clinical trials. The pathogenesis of IBD is thought to be due in part to perturbations in the gut microflora that disrupt homeostasis. Therefore, it is logical to extend the successes of FMT in CDI to the treatment of IBD. Case reports and series for the treatment of IBD by FMT have shown promise with regards to treatment success and safety despite the limitations of the reporting. While several questions remain unanswered such as the long term consequences of FMT on the recipient, this therapy may be beneficial for the treatment of UC and Crohn's disease, particularly those with concurrent CDI or with pouchitis. The study of the gut microbiome has opened an exciting new world in medicine raising as many questions as it seems to answer. It is nonetheless here to stay with additional data from randomized controlled trials much needed. Synthetic and multi-microbial stool substitutes are an inevitable advance that we are likely to see in the near future.

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Therapeutic drug monitoring in patients with inflammatory bowel disease

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Abstract

Thiopurine analogs and anti-tumor necrosis factor (TNF) agents have dramatically changed the therapeutics of inflammatory bowel diseases (IBD), improving short and long-term outcomes. Unfortunately some patients do not respond to therapy and others lose response over time. The pharmacokinetic properties of these drugs are complex, with high inter-patient variability. Thiopurine analogs are metabolized through a series of pathways, which vary according to the patients' pharmacogenetic profile. This profile largely determines the ratios of metabolites, which are in turn associated with likelihoods of clinical efficacy and/or toxicity. Understanding these mechanisms allows for manipulation of drug dose, aiming to reduce the development of toxicity while improving the efficacy of treatment. The efficacy of anti-TNF drugs is influenced by many pharmacodynamic variables. Several factors may alter drug clearance, including the concomitant use of immunomodulators (thiopurine analogs and methotrexate), systemic inflammation, the presence of anti-drug antibodies, and body mass. The treatment of IBD has evolved with the understanding of the pharmacologic profiles of immunomodulating and TNF-inhibiting medications, with good evidence for improvement in patient outcomes

observed when measuring metabolic pathway indices. The role of routine measurement of metabolite/drug levels and antibodies warrants further prospective studies as we enter the era of personalized IBD care.

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Key words: Inflammatory bowel disease; Anti-tumor necrosis factor; Infliximab; Adalimumab; Drug level; Azathioprine; Thiopurines; Antibodies; Drug monitoring; Thioguanine

Core tip: The use of thiopurine analogs and anti-tumor necrosis factor (TNF) agents in patients with inflammatory bowel diseases (IBD) has improved outcomes. The pharmacokinetics of thiopurines is variable among patients, and some do not benefit from these drugs. The manipulation and monitoring of thiopurines can potentially increase response to treatment and/or reduce the development of toxicity. The efficacy of anti-TNF drugs is also variable and several factors can modify drug clearance, including the concomitant use of immunomodulators, systemic inflammation, the presence of anti-drug antibodies, and body mass. The treatment of IBD has advanced with the understanding of the pharmacologic profiles of immunomodulating and TNF-inhibiting medications.

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INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic disor-

ders of the intestinal tract characterized by relapsing and remitting intestinal inflammation; Crohn's disease (CD) and ulcerative colitis (UC) are the best-recognized of these entities. The etiology of IBD is not clear, but it is thought to occur when the intestinal flora induces an exaggerated immune response in the context of genetic predisposition. Most therapeutic regimens focus on immunosuppression as the hallmark of treatment with medications including corticosteroids, purine anti-metabolites, and newer biologic agents that target specific molecules of the inflammatory cascade like tumor necrosis factor (TNF). Dramatic advances in the treatment of IBD have been seen with the availability of new drugs and our ability to tailor the treatment strategy for each patient. Drug monitoring is relevant not only when trying to achieve treatment efficacy, but also when trying to mitigate toxic side effects and optimize costs. In medicine, we usually measure levels in drugs with a narrow therapeutic range and that pose significant deleterious side effects with overdosing (*e.g.*, theophylline, cyclosporine, and lithium). With some antibiotics (*e.g.*, vancomycin), we measure levels in order to assure the patient receives the required dose to induce a therapeutic effect. Accordingly, the treatment of IBD has evolved with advances in laboratory medicine. We will review the latest evidence concerning measurement of genomic risk for metabolic side effects and levels of metabolites, drugs and antibodies used in the treatment of IBD, including thiopurines (purine anti-metabolites) and anti tumor necrosis factor (anti-TNF) agents.

THIOPURINE ANALOGS

In IBD patients, thiopurines have been successfully employed for decades. The thiopurine analogs with activity against IBD include mercaptopurine (MP) and its pro-drug azathioprine (AZA). The general consensus is that the thiopurine medications are effective at preventing relapse of quiescent disease, but not at inducing remission^[1]. They also exert a beneficial effect when combined with biologic drugs such as infliximab (an anti-TNF agent), improving rates of clinical remission and mucosal healing^[2].

These medications antagonize endogenous purines, interfering with the synthesis of DNA. They were first developed to treat leukemia in pediatric patients, but because of their observed anti-proliferative effect on T-cells, they have been successfully used to treat autoimmune disease and to prevent graft rejection after solid organ transplantation. While the exact molecular mechanism by which thiopurines exert their immunosuppressive effect is not well-comprehended, several theories have been proposed. One hypothesis suggests that 6-thioguanine (6-TGN), a metabolite of AZA, accumulates in lymphocytes and blocks the expression of inflammatory-related cytokines, including TNF-related apoptosis-inducing ligand, TNF receptor S7, and alpha-4-integrins, ultimately inhibiting the inflammatory response induced by T-cells

in the intestinal lamina propria in patients with IBD^[3].

Drug metabolism

The metabolism of AZA is quite complex, and involves many enzymatic pathways producing active, inactive, and potentially toxic metabolites (Figure 1). After getting absorbed from the gastrointestinal tract, 88% of AZA is converted to MP and methyl-nitro-thioimidazole in red blood cells (RBC)^[4]. MP can then be metabolized through three of the following competing pathways: conversion to 6-thiouric acid by xanthine oxidase (XO), methylation by thiopurine methyltransferase (TPMT) into 6-methyl mercaptopurine (6-MMP) (which is associated with potential hepatotoxicity), or conversion to thioinosine monophosphate (TIMP) by hypoxanthine phosphoribosyltransferase (HGPRT)^[5]. TIMP is subsequently converted into 6-TGN, which are thought to be desirable therapeutic metabolites of AZA/MP^[4,6]. Though 6-TGN is a favorable metabolite, high levels of this agent can result in life-threatening myelosuppression.

Measuring the thiopurine methyltransferase phenotype/genotype

Thiopurine drugs have a complex metabolism, and some metabolites may cause life-threatening toxicity, including myelosuppression and hepatotoxicity. TPMT catalyzes one of the rate-limiting pathways in AZA/MP metabolism. Because TPMT has variable activity among patients, checking TPMT activity prior to initiation of thiopurine therapy is routinely used in clinical practice and is recommended. TPMT induces the metabolism of MP to 6-MMP, "competing" with the HGPRT and XO enzymatic pathways (Figure 1). TPMT also metabolizes TIMP to 6-methyl-thioinosine monophosphate (6-MeTIMP); thus, low TPMT activity results in greater conversion of MP to 6-TGN, as less TIMP is converted to 6-MeTIMP. Measuring TPMT before prescribing AZA/MP can help predict those patients who will accumulate high levels of 6-TGN relative to 6-MMP, a profile which increases the risk of leukopenia early in the treatment course. The TPMT status of a patient may be identified by requesting the genotype or the phenotype from a commercial laboratory, with the phenotype testing being in general more clinically useful.

TPMT genotype: Studies in different racial and ethnic backgrounds have shown that most of the population (89%) are homozygous for the wild type (WT) *TPMT* gene (high TPMT metabolism), 10% are heterozygous for the WT and a low metabolic polymorphism (intermediate TPMT metabolism), and 1 in 300 are homozygous for low TPMT metabolic polymorphism (low TPMT metabolism)^[7]. While higher 6-TGN levels are associated with a better clinical response, they also increase risk of myelotoxicity with AZA/MP; therefore, determining TPMT phenotype/genotype is currently used to predict early leukopenia^[6]. Further research in this area has resulted in the identification of fourteen single nucleotide

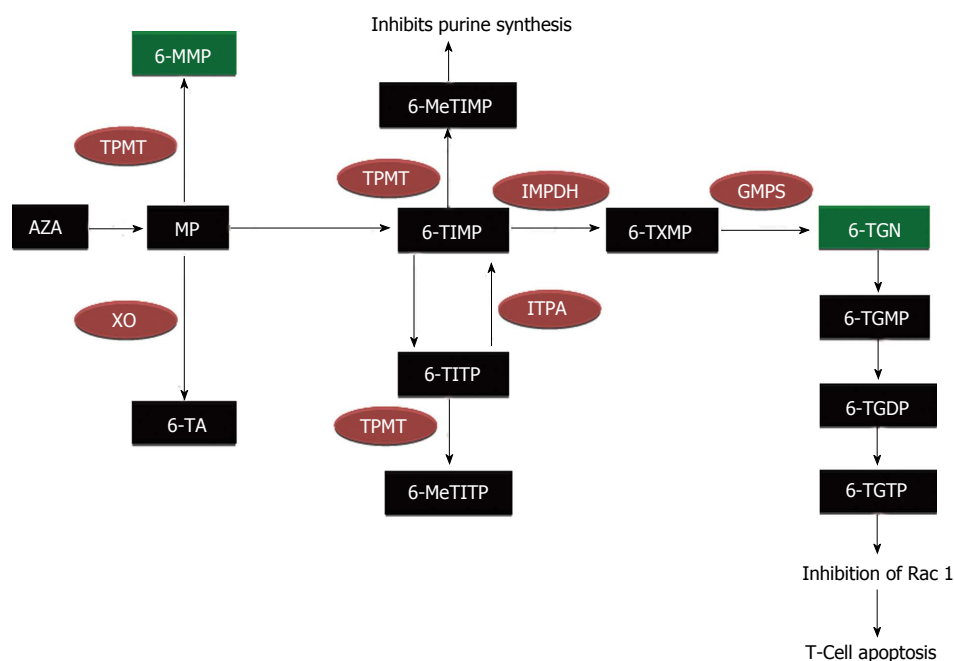


Figure 1 Metabolic pathway of azathioprine/mercaptopurine metabolism. AZA: Azathioprine; MP: Mercaptopurine; TPMT: Thiopurine methyltransferase; XO: Xanthine oxidase; IMPDH: Inosine-5-monophosphate dehydrogenase; GMPS: Guanidine- 5-monophosphate synthetase; 6-TA: 6-Thiouric acid; 6-MMP: 6-Methyl-mercaptopurine; 6-TIMP, 6-Thioinosine monophosphate; 6-MeTIMP: 6-Methylthioinosine monophosphate; 6-MeTITP: 6-Methyl thioinosine triphosphate pyrophosphate; 6-TITP: 6-Thioinosine triphosphate pyrophosphate; TXMP: Thioxanthosine monophosphate; 6-TGN: 6-Thioguanine nucleotides; 6-TGMP: 6-Thioguanine monophosphate; 6-TGDP: 6-Thioguanine diphosphate; 6-TGTP: 6-Thioguanine triphosphate.

polymorphisms for the *TPMT* gene that cause a diminished or absent enzymatic activity.

TPMT phenotype: As with genotype, enzyme activity (or phenotype) may also be measured and sub-divided into three major groups (high, intermediate, and low *TPMT* metabolizers). The correlation between *TPMT* genotype and phenotype varies between 65 and 89%^[8,9]. The cause of this variance is unclear, but measuring phenotype has a better predictive value for myelosuppression when compared to genotype^[8].

Select situations exist where the genotype can theoretically be more reliable than the phenotype. Because *TPMT* is measured in erythrocytes and uremia may affect the assay, measuring *TPMT* genotype and not phenotype may be reasonable when a patient has had a recent transfusion of red blood cells or has a high blood urea nitrogen, (usually in patients requiring dialysis)^[10]. Also, some medications including azathioprine itself and some diuretics may increase *TPMT* activity, but the clinical significance of this effect is not clear^[11]. Conversely, mesalamines and sulfasalazine inhibit *TPMT*, theoretically increasing the risk of leukopenia, though this claim is unproven^[12].

Monitoring thiopurine metabolites

Once the decision has been made to treat patients and at a particular dose, monitoring thiopurine metabolite levels is a clinical option. Measuring metabolites has two important applications, increasing the likelihood of treatment efficacy and reducing the risk of treatment-related toxicities. The two metabolites that are commercially available

are 6-TGN and 6-MMP.

6-TGN has been the metabolite most associated with treatment efficacy; as such, its measurement has been proposed as a strategy to optimize treatment in patients with IBD receiving AZA/MP. 6-TGN is a metabolite of TIMP, which goes through a series of phosphorylation events resulting in 6-thioguanine diphosphate. A 6-TGN level $> 230 \text{ pmol}/8 \times 10^8 \text{ RBC}$ has been correlated with clinical remission in both adults and children with IBD^[6,13]. Another study using a different assay that included only adult patients failed to show a relation between 6-TGN levels and clinical activity^[14].

The need to follow 6-TGN levels during treatment has not been well-established. In a prospective cohort study, Wright *et al*^[15] found that patients on a stable dose of azathioprine present with variable levels of 6-TGN over time, bringing into question the value of interpreting any single 6-TGN level. The difference in outcomes among studies is unclear, but could be related to the heterogeneity in the instrument used to determine IBD activity and the use of different assays to measure the 6-TGN levels. Another added potential use for metabolite measurement is to assess adherence to medical therapy. If both 6-TGN and 6-MMP are low, it is likely the patient is not ingesting or absorbing the medication. Randomized controlled trials looking at the role of serial measurements of thiopurine metabolites and the effect of subsequent dose adjustment on outcomes are needed.

AZA metabolite measurement can also be used to help prevent drug-related toxicity. 6-MMP is a metabolite produced from MP by *TPMT*. Higher 6-MMP levels have been found to correlate with a higher risk of hepatotoxicity.

icity. Even though patients with 6-MMP levels > 5700 pmol/ 8×10^8 RBC have a three-fold increased risk of hepatotoxicity, not all patients with a high 6-MMP level will develop elevated liver enzymes, and having a low 6-MMP level does not preclude the development of hepatotoxicity^[6,16]. As with 6-MMP, some patients with very high 6-TGN levels do not develop myelotoxicity while some with low 6-TGN levels may still develop this abnormality. Thus, measuring 6-TGN and 6-MMP levels do not replace monitoring liver enzymes and blood counts. 6-TGN level measurements can also be useful to identify those patients who will not experience clinical benefit despite an optimal AZA/MP dose. Patients with normal TPMT activity and 6-TGN levels > 400 pmol/ 8×10^8 RBC who do not achieve clinical remission will likely remain refractory to treatment even if the treatment is continued for 6 more months or the dose is increased^[17].

Patients resistant to AZA/MP therapy and the use of allopurinol

Patients who fail therapy with AZA/MP may not always benefit from dose escalation. The metabolic pathways in some patients favor an increased conversion of medication into 6-MMP rather than 6-TGN. Metabolite levels confirm this phenomenon when increasing thiopurine dose in patients who are clinically not responding^[18]. This finding supports the metabolic heterogeneity that exists among the IBD population and how some patients preferentially favor particular AZA/MP metabolic pathways. The identification of these patients represents another potential use for metabolite measurement. Up to 24% of patients preferentially produce 6-MMP while maintaining low levels of 6-TGN despite increasing the dose^[18]; these patients usually have high TPMT activity, which is genetically determined and are usually labeled as “thiopurine resistant”.

In this setting, one option is to switch to another therapeutic class (methotrexate or anti-TNFs), but manipulation of the known pathways provides an alternative option to optimize therapy in these so-called “shunters”. Inhibition of TPMT could reduce the production of 6-MMP, but drugs with this effect (*e.g.*, mesalamines/sulfasalazine) have failed to reliably optimize the metabolic profile, likely due to their weak inhibitory effect on the enzyme. Another potential target is XO. This enzyme converts MP into 6-thiouric acid, which has no known biologic effect. When this enzyme is inhibited, much of the MP is shunted down the 6-TGN pathway. Allopurinol, a XO-inhibitor used in the treatment of gout, has been successfully used to shunt metabolism of AZA/MP down this more favorable 6-TGN pathway. The mechanism by which this occurs is not completely understood, as the sole inhibition of XO would not explain the rise in 6-TGN. Sparrow *et al*^[19] selected a group of patients who had a preferable metabolism towards 6-MMP and initiated therapy with 100 mg of allopurinol while reducing the AZA/MP dose by 25%-50%. After 2-4 wk on this regimen, they found that the level of 6-TGN had significantly increased while the 6-MMP level had decreased. A

subsequent study by the same group looking at clinical response in AZA/MP non-responders when starting allopurinol found that the allopurinol-immunomodulator regimen also improved disease activity^[20]. This beneficial effect was not only observed for disease activity, but also reversed hepatotoxicity, presumably by reducing the 6-MMP levels.

However, there are several issues that must be addressed when starting patients on a combination of a thiopurine and allopurinol. First, we recommend serial blood counts, checking a complete blood count (CBC) with differential weekly for the first month and monthly thereafter. Thiopurine metabolites may be checked one month after initiating therapy in order to ensure the goal of increased 6-TGN and decreased 6-MMP is achieved. If leukopenia develops, the dose of AZA/MP can be reduced.

Another proposed strategy to overcome the shunting of MP towards 6-MMP is splitting the thiopurine into twice daily dosing. A retrospective study in patients with preferential 6-MMP metabolism showed that dividing the daily dose of the thiopurine (MP or AZA) modifies how the drug is metabolized, resulting in a significant reduction in 6-MMP levels and decreasing toxicity without having an adverse impact on clinical disease activity or 6-TGN levels^[21]. The exact mechanism explaining the beneficial outcomes seen when implementing this strategy is unknown, and further studies are needed to confirm the results.

In summary, measuring TPMT activity prior to thiopurine initiation has improved the clinical care for IBD patients, both by improving treatment efficacy and by ameliorating iatrogenic toxicities. Many clinicians, including our group, also follow thiopurine metabolite levels with the intent of positively impacting patient care. The enclosed algorithm represents a logical approach to the care of IBD patients on thiopurine therapy (Figure 2).

ANTI-TUMOR NECROSIS FACTOR

Anti-TNF agents have dramatically changed the management of CD and UC. Though four anti-TNF agents are currently approved to treat IBD, we will focus on infliximab (IFX) and adalimumab (ADA), as these are the two anti-TNF drugs with commercially available assays to measure drug levels and anti-drug antibodies. IFX is a chimeric IgG1 (human-constant and murine-variable regions) monoclonal antibody consisting of human constant and murine variable regions; it is indicated for induction and maintenance of clinical remission in patients with moderate-to-severely active CD and UC^[22,23]. ADA is a recombinant fully human IgG1 monoclonal antibody, also approved for the induction and maintenance of remission in patients with CD and UC^[24-26].

These biologic therapies are of proven benefit, reducing rates of hospitalization and surgery rates while improving quality of life^[27,28]. Unfortunately, up to 50% of patients lose response to treatment (secondary non-responders) and up to 30% do not respond at all (primary

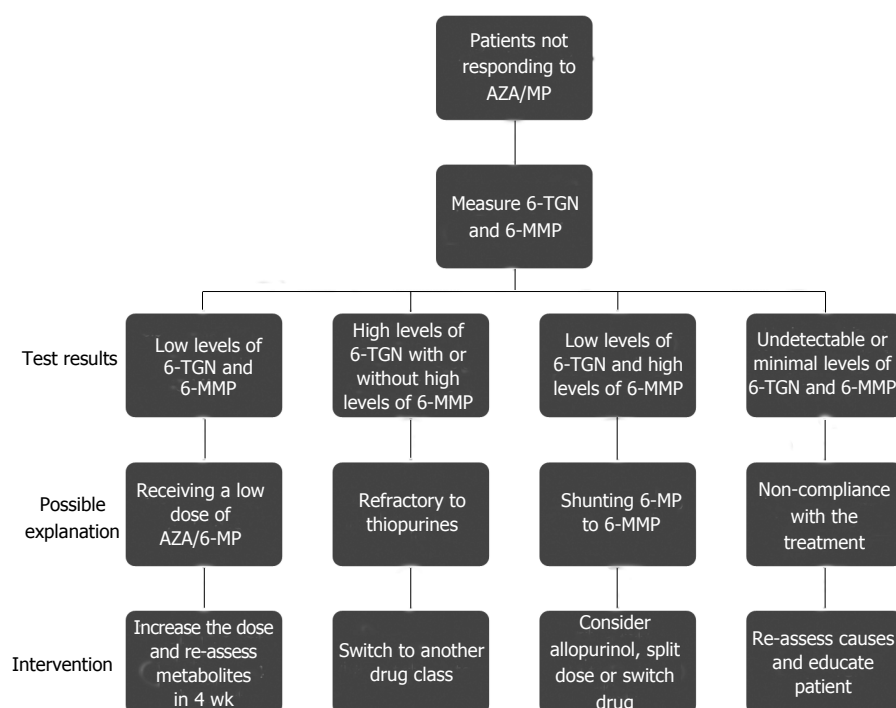


Figure 2 Algorithm with recommendation on how to manage patients that fail therapy with Azathioprine/Mercaptopurine. AZA: Azathioprine; MP: Mercaptopurine; 6-TGN: 6-Thioguanine nucleotides; 6-MMP: 6-Methylmercaptopurine.

non-responders)^[29]. The rationale for lack or loss of response is multi-factorial, but is likely related to the molecular structures and complex pharmacokinetics (PK) and pharmacodynamics of the medications, including the development of anti-drug antibodies. One of the proposed strategies when patients lose response to an anti-TNF is switching to another drug in the same or different class. Another strategy is to empirically increase the dose, hoping to overcome increased drug clearance. Unfortunately, this can lead to significant adverse events including hypersensitivity reactions. A third potential tactic is to measure drug levels and antibodies, trying to identify those patients who will benefit from dose escalation and those who will be best served by switching to an alternate drug class. This latter strategy has been proven to be more cost effective when compared to the former and highlights the importance and usefulness of measuring levels in these patients^[30]. Most of our collective experience has been measuring IFX levels and antibodies to IFX (ATI), but the measurement of ADA and antibodies to ADA (ATA) has recently become available commercially.

PHARMACOKINETICS OF ANTI-TNF

Within an individual patient, a linear relationship exists between anti-TNF dose and serum medication levels^[31]. However, inter-patient PK variability complicates the reliable prediction of medication levels among patients^[32,33]. Some have theorized that this inconsistency occurs as a result of differences in body mass, but variable levels are observed with drugs with weight-based dosing like IFX. The clearance of TNF-inhibitors likely also plays a role.

Anti-TNFs are monoclonal antibodies with a high molecular weight, and as is the case with other with IgGs, they are metabolized through many pathways. One of

the primary mechanisms by which anti-TNFs are cleared involves the reticuloendothelial system (RES). The main component of IgG metabolism is the Brambell receptor (FcRn), which is responsible for internalizing an antibody intra-cellularly, degrading the antigen, and then “recycling” it by releasing it back into the circulation for excretion^[31]. High circulating IgG levels may saturate the FcRn, translating into an inverse association between IgG levels and anti-TNF clearance.

Many of the mechanisms underlying variation in drug levels and clearance of TNF-inhibitors are summarized in Figure 3.

Anti-drug antibodies

The human immune system recognizes these biologic drugs as foreign antigens, creating specific antibodies that neutralize their effect. This phenomenon, known as immunogenicity, increases drug clearance and ultimately may contribute to treatment failure. Immunogenicity was first described with IFX, a chimeric antibody. Originally, it was believed that ADA would demonstrate less immunogenicity as it is a fully human antibody, but this theory was not substantiated by clinical practice findings^[33,34].

In patients with IBD, the negative effect that antibodies to IFX (ATI) and antibodies to ADA (ATA) have on clinical outcomes has been well established^[32,35-38]. ATI and ATA bind to the medication, forming an immune complex that induces drug clearance via the RES. The ability to routinely measure drug levels and anti-drug antibodies has led to an understanding of why some patients lose response to therapy.

Currently, there are several methods to measure IFX and ADA. Most studies have been performed using a solid-phase, double-antigen, enzyme-linked immunosorbent assay (ELISA). Unfortunately, this assay cannot measure

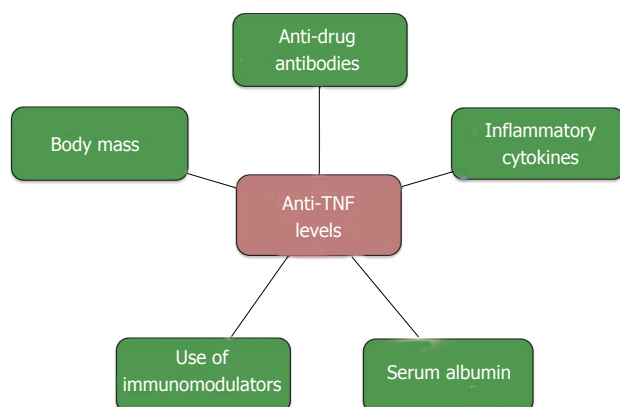


Figure 3 Variables associated with Anti-tumor necrosis factor levels. TNF: Tumor necrosis factor.

the presence of antibodies when there are detectable levels of anti-TNF. Another method is a fluid-phase radioimmunoassay, which can report antibodies irrespective of the presence of drug. A recently developed assay is a homogeneous mobility shift assay using size-exclusion high-performance liquid chromatography, which has a high sensitivity and specificity and can potentially detect all isotypes of immunoglobulin^[39]. This modality permits the measurement of antibodies to anti-TNF medications, even in the presence of detectable drug. However, most clinical studies have used the ELISA assay, which could have implications in how we interpret the available data.

Role of immunomodulators with biologic therapy

Observations from randomized controlled trials have shown that the concomitant use of immunomodulators (AZA, MP or methotrexate) and biologic medications increases anti-TNF levels^[36]. It is generally believed that this occurs by diminishing immunogenicity (decreasing the formation of anti-TNF antibodies) while simultaneously reducing the amount of systemic inflammation which, as we describe below, will negatively affect IgG levels.

The presence of ATI has consistently been associated with lower IFX levels^[36]. In an exploratory analysis of a randomized controlled trial looking at efficacy of IFX monotherapy, AZA monotherapy, and the two biologic drugs combined with immunomodulators, the authors found that at week 30 the IFX levels were 1.6 µg per milliliter for patients in the IFX group and 3.5 µg per milliliter for those in the combination therapy group ($P < 0.001$)^[40]. They also found that only 1 of 116 patients (0.9%) receiving combination therapy (compared to 15 of 103 patients (14.6%) receiving IFX monotherapy) had ATI^[40]. Studies looking at the effect of ATA on ADA levels have yielded similar results^[33,37].

Even though most studies have shown a clear association between the presence of anti-TNF antibodies and drug levels, some data have failed to support this observation. Lichtenstein *et al* looked at the influence that concomitant immunomodulators and IFX therapy had on drug levels^[41]. They reviewed the ACCENT I and II trials (effect on induction and maintenance of remission

that IFX have in CD) as well as ACT 1 and 2 (effect on induction and maintenance of remission that IFX have in UC). In contrast to the previously mentioned studies, they found that IFX levels were similar when comparing patients who did and did not receive immunomodulators, even though those patients that received immunomodulators had a higher incidence of ATI antibodies to infliximab^[41]. Of note, this report was based on a post-hoc analysis and the results should be interpreted in that context.

Disease activity and systemic inflammation

Patients with severe disease have been found to have lower levels of anti-TNF. The exact mechanisms by which disease activity and systemic inflammation affect drug clearance are not completely understood. Systemic inflammation induces the RES to increase its catabolic activity, reducing levels of IgG and albumin. Direct and indirect markers of inflammation have been used to predict IFX efficacy. For example, higher levels of CRP correlate with poor response to IFX and may predict loss of response^[32,42,43]. Also, low albumin levels are associated with lower IFX levels, which suggests that increased catabolic activity in systemic inflammation induces the Brambell receptor^[44]. As with IFX, preliminary studies looking at ADA levels showed that higher CRP levels are associated with lower ADA levels^[33,34]. In one study, a minimum ADA cut-point of 5 µg/mL best predicted elevation of CRP levels^[33].

These studies cannot prove causation but do show an association between low drug levels, high CRP, and low albumin. Severe inflammation of the mucosa also induces a protein-losing enteropathy, with a loss of drug through the intestinal lumen. A recent study that included patients going through IFX induction therapy found that even though all patients had detectable IFX in the stool, those who did not respond to treatment had a significantly higher level of fecal IFX^[45].

Body mass and composition may influence anti-TNF drug levels

Many other variables have been proposed to affect the levels of anti-TNF. One study found that body mass and gender influence the central volume of distribution of IFX (increasing with weight and male gender)^[46]. While one might suspect these results were due to the collinearity of gender and mass (as men weigh more than women), the authors tested each variable individually and concluded that each had independent influence on the central volume of distribution; this finding was attributed to the fact plasma volume is lower in women than in men. The influence of body mass on ADA levels in patients with IBD is unknown, but might play a key role as ADA does not have weight-based dosing; this also applies to other anti-TNFs approved for IBD (certolizumab pegol and golimumab).

An important distinction must be made between total body mass and body composition. For example, Bultman *et al*^[47]

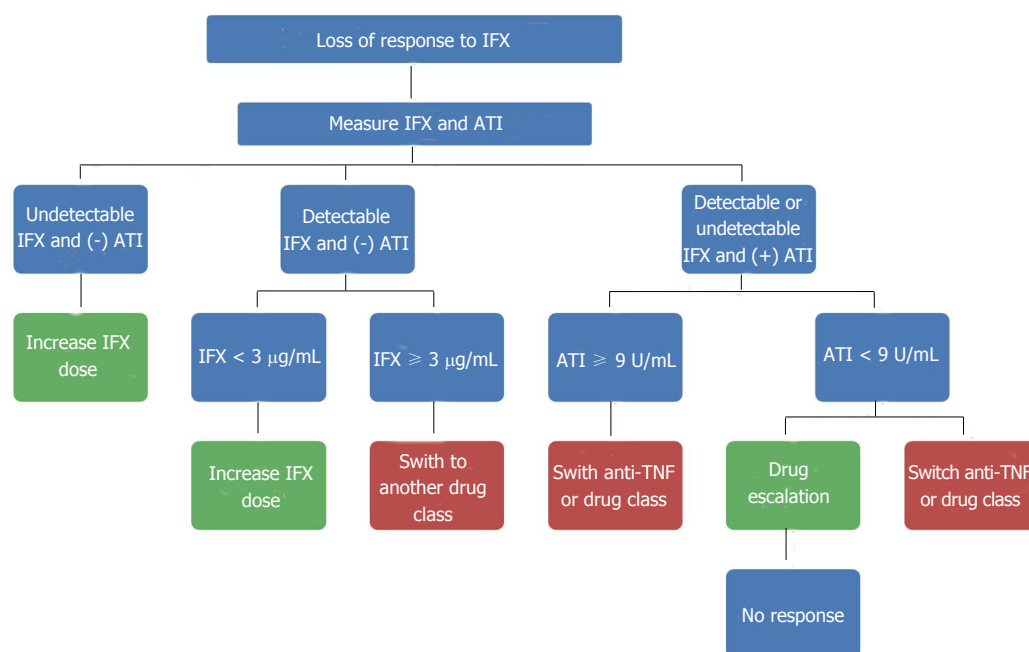


Figure 4 Approaches to patients on infliximab in the setting of loss of response. ATI: Anti-infliximab antibody; IFX: Infliximab; TNF: Tumor necrosis factor.

found that patients on ADA who required dose escalation had a higher body mass index (BMI) despite equivalent dosing of ADA given per kg of body weight. The component responsible for the increased BMI is unclear, but it may be related to mesenteric fat, an important contributor to the inflammatory response in CD^[48].

CLINICAL IMPLICATIONS OF MEASURING ANTI-TNF LEVELS AND ANTIBODIES

Many studies have shown that higher IFX levels are associated with better outcomes in patients with IBD. A detectable IFX trough level is associated with a higher rate of clinical remission, lower serum CRP levels, and an improvement in endoscopic disease activity^[32,49].

The presence of ATI has been associated with treatment failure and lower IFX levels^[36]. In a landmark study, Baert *et al*^[35] studied the immunogenicity of a group of patients with CD. They found that 61% had detectable ATI after the 5th infusion, which did not increase after subsequent infusions. This high prevalence of ATI can be explained by the fact those patients received episodic dosing (when they relapsed) and not scheduled (every 8 wk) infusions. They found that those patients with ATI >8.0 µg/mL before an IFX infusion had a shorter duration of response and a higher risk of infusion reactions^[35]. Afif *et al*^[50] looked at the clinical utility of measuring IFX levels and ATI in patients with loss of response or an incomplete response to IFX. They found that most patients with ATI did not respond to IFX dose escalation, but those with no ATIs and sub-therapeutic concentrations did benefit from a higher dose^[50].

With ADA, the experience is more limited. In an observational study, Karmiris *et al*^[37] found that lower

ADA serum trough levels were associated with drug discontinuation and the presence of ATA was associated with lower ADA levels. Recent studies have found that detectable ATA and ADA levels < 5 µg/mL are associated with higher CRP serum levels, increased endoscopic inflammatory activity, and use of steroids^[33,34]. Another recent study measuring ADA trough levels by ELISA assay showed similar results; an ADA trough of 4.85-4.90 µg/mL was the optimal cut-off value for predicting clinical remission and mucosal healing^[51].

ROLE OF ANTI-TNF DRUG MONITORING IN CLINICAL PRACTICE

Commercial assays to measure anti-TNF drug and antibody levels have been available for a relatively short time; direct cost implications for patients and/or insurers have limited their widespread clinical use despite documented efficacy in directing patient management. Knowledge of drug levels and measurements of anti-drug antibodies can help identify those patients who will benefit from dose escalation versus those who are unlikely to respond to this strategy (high titers of anti-drug antibodies or those with high drug levels but persistent intestinal inflammation). A recent study modeled both strategies and found that testing for drug levels and antibodies in patients with secondary loss of response is more cost-effective when compared to empiric drug escalation^[52].

The titer of ATI may also predict response to dose escalation, as demonstrated by a cohort of 90 patients undergoing serial IFX and ATI levels with at least one ATI positive measurement throughout the follow-up period. In 15 of those patients (28%), ATI disappeared overtime^[53]. Only 2 of those 15 patients (13%) required

discontinuation of therapy, which is quite significant as ATI may be transient and do not necessarily lead to treatment failure. However, they also found that ATI > 7.95 U/mL was associated with IFX discontinuation, which may suggest that patients with high ATI titers would not benefit from dose adjustment^[53]. Most of the available evidence includes patients on IFX, so further studies are needed with the other medications. A proposed algorithm to guide therapy in patients receiving IFX who lose response to treatment is shown in Figure 4.

An unanswered question is the potential benefit of measuring drug levels in all patients and adjusting the dose accordingly to maintain adequate levels, thereby avoiding immunogenicity. Considering low trough IFX levels are associated with immunogenicity and the fact that the dose of IFX and serum levels are not always linear, measuring drug levels even in patients with clinical response could improve outcomes. The study cited above also showed that an IFX trough level at week 14 of < 2.2 µg/mL correlated with IFX discontinuation^[53]. These results are based on a retrospective analysis and future prospective controlled studies are needed, especially considering anti-TNF levels are influenced by several variables that can fluctuate over time and among individuals.

The repeated demonstration of clinical utility and evidence for cost-effective patient care have made routine measurement of anti-TNF drug and antibody levels a regular part of our group's clinical practice. For UC, when patients tend to have a lower starting albumin level and a markedly elevated CRP, our practice has been to give IFX at a higher dose (10 mg/kg) and then check levels after induction. While this practice has yet to be validated, we have had anecdotal success in achieving remission in patients who we feel clinically merit higher induction doses. Monitoring trough drug levels after induction allows for a tapered return to lower doses and a sustained remission.

CONCLUSION

The pharmacokinetic and pharmacodynamic properties of anti-TNFs and thiopurines are complex. Considerable inter-individual variability exists due not only to differences in pharmacogenetic factors but also disease phenotype and concomitant drug therapy. Measuring drug levels, metabolites, and anti-drug antibodies may help overcome treatment failures while mitigating toxic side effects, but further randomized studies to confirm these findings are needed. The treatment of IBD has evolved with the understanding of the pharmacologic profiles of immunomodulating and TNF-inhibiting medications, with good evidence for improvement in patient outcomes observed when measuring metabolic pathway indices. The role of routine measurement of metabolite/drug levels and antibodies warrants further prospective studies as we enter the era of personalized IBD care.

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Advanced therapeutic endoscopist and inflammatory bowel disease: Dawn of a new role

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Abstract

Endoscopy plays a key role in the diagnosis and treatment of patients with inflammatory bowel disease (IBD). Colonoscopy has been traditionally used in the diagnosis of IBD and helps in determination of an important end point in patient management, "mucosal healing". However, the involvement of an advanced endoscopist has expanded with innovations in therapeutic and newer imaging techniques. Endoscopists are increasingly being involved in the management of anastomotic and small bowel strictures in these patients. The advent of balloon enteroscopy has helped us access areas not deemed possible in the past for dilations. An advanced endoscopist also plays an integral part in managing ileal pouch-anal anastomosis complications including management of pouch strictures and sinuses. The use of rectal endoscopic ultrasound has been expanded for imaging of perianal fistulae in patients with Crohn's disease and appears much more sensitive than magnetic resonance imaging and exam under anesthesia. Advanced endoscopists also play an

integral part in detection of dysplasia by employing advanced imaging techniques. In fact the paradigm for neoplasia surveillance in IBD is rapidly evolving with advancements in endoscopic imaging technology with pancolonoscopic chromoendoscopy becoming the main imaging modality for neoplasia surveillance in IBD patients in most institutions. Advanced endoscopists are also called upon to diagnose primary sclerosing cholangitis (PSC) and also offer options for endoscopic management of strictures through endoscopic retrograde cholangiopancreatography (ERCP). In addition, PSC patients are at increased risk of developing cholangiocarcinoma with a 20% lifetime risk. Brush cytology obtained during ERCP and use of fluorescence in situ hybridization which assesses the presence of chromosomal aneuploidy (abnormality in chromosome number) are established initial diagnostic techniques in the investigation of patients with biliary strictures. Thus advanced endoscopists play an integral part in the management of IBD patients and our article aims to summarize the current evidence which supports this role and calls for developing and training a new breed of interventionalists who specialize in the management of IBD patients and complications specific to those patients.

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Key words: Inflammatory bowel disease; Endoscopy; Therapeutic endoscopy; Primary sclerosing cholangitis

Core tip: Endoscopy plays a key role in the diagnosis and treatment of patients with inflammatory bowel disease. The involvement of an advanced endoscopist has expanded with innovations in designs of endoscopes and newer imaging techniques. Our article aims to summarize the current evidence which supports the role of an advanced endoscopist in the management of colonic and ileal pouch strictures, biliary strictures in patients with primary sclerosing cholangitis, endoscopic

diagnosis of colonic fistulae and surveillance of colon neoplasia and cholangiocarcinoma.

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INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are a group of inflammatory bowel diseases (IBD) that have environmental, immunological and bacterial etiologies. The role of an endoscopist has been well defined in the initial diagnosis of these disorders, assessment of disease severity and differentiation between the two disease processes. Previous studies have reported that in 80% of CD patients, at last one surgical resection will be required within 10 years of CD diagnosis^[1,2]. Although surgical treatment is effective for CD strictures, there is invariably a high risk of recurrence of CD which may result in repeat surgery in up to 34% of patients^[1,3]. Repeated surgery can result in complications related to short bowel syndrome, requirement for total parenteral nutrition and its attendant complications. The advent of endoscopy in the management of complicated CD strictures has changed the approach to the management of anastomotic and small bowel strictures in these patients. The advent of balloon enteroscopy has helped us access areas not deemed possible in the past for dilations. An advanced endoscopist also plays an integral part in managing ileal pouch-anal anastomosis (IPAA) complications including management of pouch strictures and sinuses. Colorectal cancer (CRC) is a serious potential complication of IBD. Advanced endoscopists play an important role in detection of dysplasia by employing advanced imaging techniques to identify early and subtle neoplastic lesions^[4].

In addition, primary sclerosing cholangitis (PSC) a chronic, cholestatic disorder is seen in 2.4%-7.5% of patients with UC^[5] and about 3.4% of patients with CD^[6]. Advanced endoscopists are called upon to diagnose PSC and also offer options for endoscopic management of strictures through endoscopic retrograde cholangiopancreatography (ERCP). In addition, PSC patients are at increased risk of developing cholangiocarcinoma (CCA) with a 20% lifetime risk^[7,8]. Brush cytology obtained during ERCP and use of fluorescence in situ hybridization (FISH) which assesses the presence of abnormality in chromosome number are established initial diagnostic techniques in the investigation of patients with biliary strictures^[9]. The aim of our review is to highlight the current evidence which supports the role of an advanced endoscopist in the management of IBD and complications specific to them.

For the purpose of this article, we will discuss the role of an advanced endoscopist under various sections.

Figure 1 illustrates the various roles of an advanced endoscopist in managing inflammatory bowel disease patient.

Inflammatory bowel strictures

The Vienna Classification describes three distinct groups of CD: inflammatory, stricturing and penetrating. It also demonstrated an association between location and disease behavior. Stricturing disease is predominant in the terminal ileum and ileocolonic locations^[10]. Subsequent studies using this classification have confirmed that most patients over the course of time will develop a penetrating or stricturing complication with stricture formation being the second most common and that this change is governed by location of disease^[11,12]. Data from the TREAT (the Crohn's therapy, resource, evaluation, and assessment tool) registry suggested factors associated with stricture formation. These were CD severity at the time of event onset (HR = 2.35, 95%CI: 1.35-4.09); CD duration (HR = 1.02, 95%CI: 1.00-1.04); ileal disease (HR = 1.56, 95%CI: 1.04-2.36); and new corticosteroid use (HR = 2.85, 95%CI: 1.23-6.57)^[13].

Strictures in CD can occur *de novo*, at sites of bowel anastomosis or in the ileal pouch. Strictures are believed to be either inflammatory or fibrotic^[13]. Inflammatory strictures have the option of being treated by medical therapy. Even then, when these patients are followed, 64% of these patients require surgery. CD patients with ileocolonic disease do worse with medical therapy ($P = 0.026$) and may require surgery sooner than patients with ileal disease ($P = 0.023$)^[14]. The fibrotic strictures are largely treated surgically with either intestinal resection or strictureplasty^[15]. Although the latter has the advantage of preserving bowel length, it has still been associated with a significant operative recurrence rate of 34% during a median follow up period of 7.5 years^[16]. These results seem comparable to those from other review studies^[17,18], one of them a meta-analysis^[17], in which surgical recurrence rates have been cited as 24% (median surgical rate at 46 mo) and 23%. Younger patients tend to run an aggressive course with a shorter duration to reoperation^[16]. Moreover, an 82% rate of second operation has been reported with upto 33% patients requiring more than 2 surgeries leading to risk of developing short bowel syndrome^[19]. This means that avoidance of repeated surgeries is an important factor in considering alternative therapies. Endoscopic balloon dilation has been one such treatment alternative. Additionally, endoscopic balloon dilatation could be considered an adjunct to surgery given that it has been shown to add at least 50% efficacy to the initial surgery by prolonging the surgery free period. This was deduced after comparing interval of 6 years from surgery to first endoscopic dilation with the post dilatation surgery free period of 3 years^[20]. It also has the advantages of reduced invasiveness and bowel preservation.

Endoscopic balloon dilation

There have been several studies aimed at reporting the clinical efficacy, technical feasibility and short and long

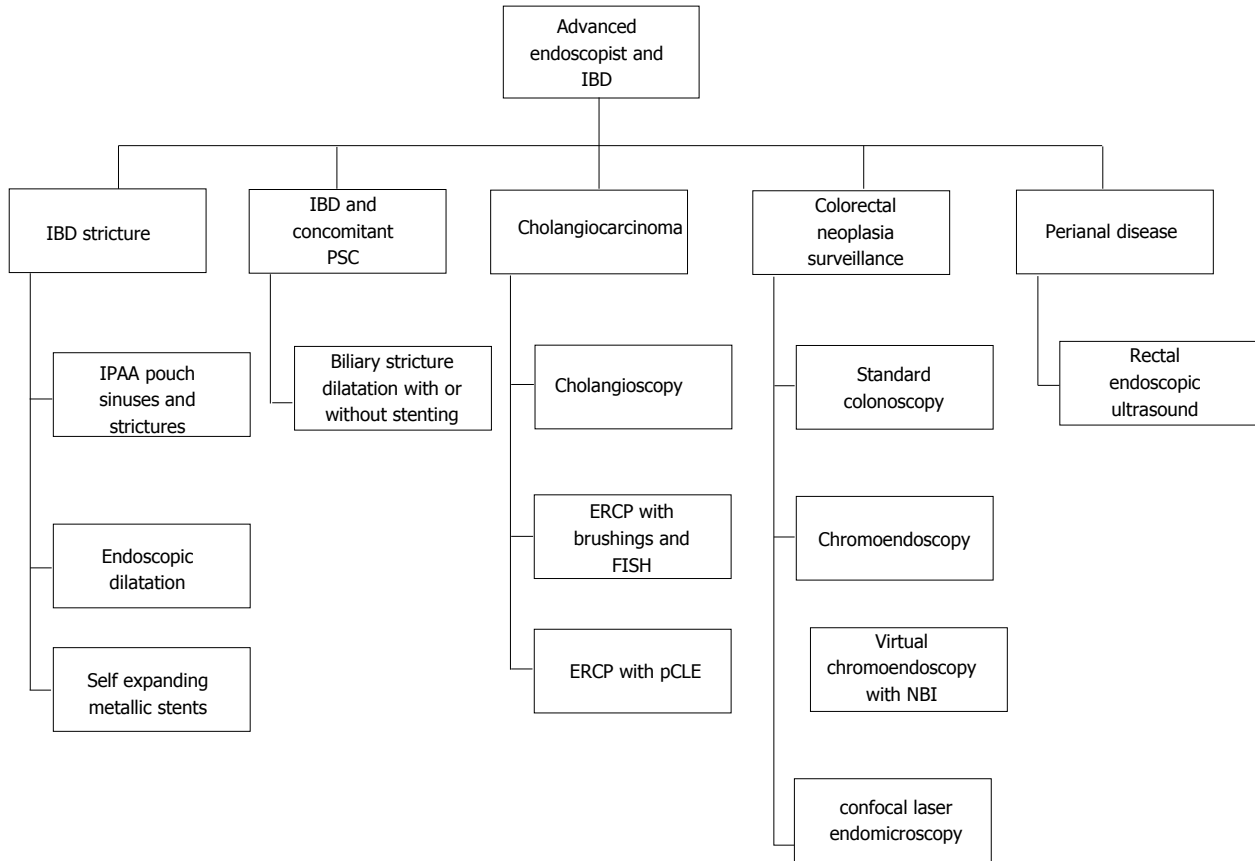


Figure 1 Figure illustrates the various roles of an advanced endoscopist in managing inflammatory bowel disease patient. IBD: Inflammatory bowel disease; IPAA: ileo-pouch anal anastomosis; PSC: Primary sclerosing cholangitis; ERCP: Endoscopic retrograde cholangiopancreatography; FISH: Fluorescence *in situ* hybridization; pCLE: Probe based confocal laser endomicroscopy; NBI: Narrowband imaging.

term results of endoscopic balloon dilatation. Almost all studies have used resolution of symptoms and/or surgery free period as outcomes. A systemic review summarized the results of important studies^[20]. Most studies included had less than 60 patients. Most of them included post-surgical strictures. The meta-analysis clearly highlighted the drawbacks with making clear conclusions from the literature because of varying caliber of endoscopic balloons used for dilation from 18 to 25 mm, different approaches that were used, number of dilatations in the same endoscopic sessions and heterogeneity concerning the duration of each single dilatation^[20]. Overall, technical success was achieved in 86% patients. The complication rates were < 5% in all studies barring two which reported a higher complication rate^[21,22]. The reasons for this have been unclear, aggressive dilatation in one of them^[21] may have been a contributing factor. The only factor that significantly affected dilatation efficacy and surgery-free follow up was length of stricture. Naïve *vs* postsurgical, steroid injection, active *vs* inactive CD were all deemed to be non significant^[20]. Importantly, endoscopic dilatation was successful in avoiding surgery at the end of the follow-up in 112 of the total 347 (67%) patients that were included in this review^[20]. If the patients who had failed for technical reasons were excluded, the success rate measured by avoidance of surgery was up to 78%^[20].

Subsequent to the meta-analysis, more studies have

been published on the efficacy of endoscopic dilation and have shown similar short (51%-89%) and long (52%-89%) term success^[23-28]. Most strictures included in these studies have been anastomotic with the exception of the Mueller *et al*^[26] study, in which 69% had *de novo* strictures. The study by Gustavsson *et al*^[27] is the largest study to date, including a total of 178 patients, and the one with the longest follow-up period (median 12 years). Most patients had either ileal or ileocolonic disease, and approximately 40% had stricturing disease at presentation. Most of the patients (80%) had anastomotic strictures. There was no difference in efficacy based on the etiology of their strictures whether anastomotic or *de novo*. Bowel perforation occurred in 1.4% of patients and use of 25 mm balloon was associated with a 9.3% complication rate, as compared to 3.5% for the other sizes ($P < 0.01$).

Another prospective single center study from Germany of 55 patients, with 74 symptomatic strictures reported their efficacy^[26]. Majority of patients in this study (69%) had *de novo* strictures. There was a 95% initial success rate, and 76% patients never required repeat treatment over the period of follow-up. 24% patients did eventually receive surgery over the follow-up period. Stricture length was the main factor that predicted the need for surgery. Interestingly, stricture location at ileocecal valve and stricture associated with fistula were significant predictors of

a negative outcome^[28]. Smoking has been reported to be a significant patient variable that negatively affects surgery free period^[29,30] and doubles the risk of recurrent stricture formation requiring a new dilatation after the first one ($P = 0.022$)^[31].

Given the high rate of stricture recurrence after dilation, intralesional injection of medications after dilation has been studied. A pilot study comparing intralesional steroid injection after balloon dilatation vs placebo did not find a reduction in time to redilatation^[32]. This is in contrast to a pediatric study of 29 patients that reported a significant trend of patients who did not receive intralesional steroids towards redilatation and surgery^[33]. Effect of intralesional injection of infliximab has been studied in small number of patients but consensus is lacking. Thienpont *et al*^[34] reported no significant effect of active disease at the time of dilatation or systemic medical therapy afterwards on redilatation or surgery. Thus, there is no clear evidence at this time to support the role of intralesional injection of medications following dilation.

We routinely perform endoscopic dilation with a 16-, 17-, and 18-mm through the scope (TTS) balloon (Boston Scientific, Inc, Boston, MA) or a 18-, 19-, and 20-mm TTS balloon with guidewire assistance. If possible, retrograde dilation with passage of the endoscope beyond the stricture and introduction of the balloon and pulling the endoscope backward and dilating the stricture is preferred.

Endoscopic balloon dilation with stenting

Metal stents have been reported as an effective alternative to surgery for the palliation of patients with colorectal neoplastic obstruction. Data regarding their use in treating benign naïve or postsurgical strictures in CD is limited and controversial. Recent studies that have used self expanding metallic^[35,36] and biodegradable^[37-39] stents have reported a high incidence of migration^[37,39]. On the other hand clinical success has ranged from 45% to 80%. The majority of stents in these studies were placed in postsurgical strictures.

Overall, efficacy of endoscopic dilatation in the treatment of small and large bowel strictures is promising with an acceptable rate of complications. Length of stricture and location of stricture are important considerations. We have not employed stenting in the management of IBD related strictures. We prefer the use of needle-knife for strictures which are refractory to balloon dilation for management.

Strictures in IPAA

The role of an advanced endoscopist in managing IPAA complications mainly caters to strictures and sinuses. IPAA strictures can occur at the pouch inlet, outlet, afferent limb or pouch body. An 11-year-experience with 1005 patients after restorative proctocolectomy and IPAA reported stricture formation in 14% of patients of which the majority (97.9%) were successfully treated with digital or bougie dilatation and only 2.1% required surgery^[40]. Another large retrospective study with 1884 patient re-

ported a similar incidence (11.2%) of stricture formation but a much higher (12%) rate of surgery to salvage pouch function. Dilatation of non fibrotic strictures was more successful than fibrotic strictures ($P = 0.0001$)^[41]. A more recent study in which a cumulative of 646 strictures were dilated reported that 87.3% patients over a median follow up of 9.6 years were able to retain their pouches. It concurred that endoscopic dilatation of pouch strictures is efficacious and safe with a low rate of complications when attempted by an experienced endoscopist^[42].

Pouch sinus is typically a late presentation of an initial anastomotic leak. The most common location of a pouch sinus is the pouch-anal anastomotic site at the presacral space. Presenting symptoms include perianal pain, pelvic pressure/discomfort and/or evidence of pelvic sepsis or pouchitis, CD of the pouch, or refractory cuffitis; others may be asymptomatic. Sinus opening and sinus tract can be detected by a combined application of pouchoscopy, contrast pouchogram, examination under anesthesia and pelvic magnetic resonance imaging (MRI). Treatment usually includes incision and drainage of the chronically infected superficial sinuses^[43]. Fibrin glue injection of the sinus may be attempted^[44]. Patients with a long sinus track who fail to heal are suitable for redo pouch.

An alternative strategy that has only recently been introduced and studied at our institution is endoscopic needle knife therapy for pouch sinuses. A total of 65 patients with pouch sinuses were treated with needle-knife therapy of which 84.6% achieved a complete or partial response. Duration from colectomy to needle knife treatment and complex nature of sinuses were inversely proportional to healing of sinuses^[45].

Endoscopic ultrasonography in the evaluation of CD fistulae

Approximately, 25% of all patients with CD develop a perianal fistula, with fistulae more frequent in patients with involvement of the rectum^[46,47]. Identification of fistulae is difficult with digital rectal examination alone or even with exam under anesthesia (EUA) because perianal disease is associated with induration and scarring. Endoscopic ultrasonography (EUS) has been used in this setting to help in the evaluation of CD fistulae.

Rectal EUS is performed by introducing a radial probe into the distal rectum and anal canal while the patient is in the left lateral position^[48]. On EUS, the fistulae in the setting of CD appear as hypoechoic structures, but the presence of air or gas in the fistula tract may make the tract internally hyperechoic^[48]. An abscess in the setting of CD fistulae can also be visualized as an anechoic or hypoechoic mass in the perianal region^[48]. Some investigators have used hydrogen peroxide injection into the cutaneous fistula site to enhance visualization as it creates bubbles that appear hyperechoic and thus make the fistula easier to identify.

The accuracy of rectal EUS in the evaluation of perianal disease has been demonstrated in 3 prospective, blinded studies^[49-51]. One of these studies compared EUS to computerized tomography (CT) in 25 patients with

suspected perianal CD^[49]. Confirmation of fistulae was done at the time of surgery. EUS was found to be more accurate than CT in the evaluation of perianal fistulae (82% *vs* 24%)^[49]. In another study, rectal EUS was compared with pelvic MRI and EUA in 22 patients with CD and perianal fistulae. Rectal EUS was found to be the most sensitive modality for imaging (82%) when compared with pelvic MRI (50%)^[50]. Rectal EUS in this study was performed with only a 7-MHz linear scanning probe. Another study comparing EUS to MRI found both to be equally accurate in the assessment of CD perianal fistulae (91% *vs* 87%)^[51].

Studies have also used EUS to monitor fistula healing and/or guide treatment. A small, randomized, prospective study showed a benefit to the use of EUS monitoring for fistula healing^[52]. Thus, it seems that a team of colorectal surgeons and advanced endoscopists seem to be the future in the evaluation and management of perianal CD.

IBD with concomitant primary sclerosing cholangitis

PSC is a chronic, cholestatic liver disease characterized by inflammation and fibrosis of both intrahepatic and extrahepatic bile duct leading to the formation of bile duct strictures. An advanced endoscopist is integral in diagnosing PSC. Although magnetic resonance cholangiopancreatography (MRCP) is the initial preferred non-invasive technique to diagnose PSC, often ERCP is required to confirm diagnosis and rule out a dominant stricture. An advanced endoscopist is often called upon to manage patients with IBD and PSC who have concomitant dominant biliary stricture in the setting of PSC.

A “dominant stricture” has been defined as a stenosis with a diameter of 1.5 mm in the common bile duct or of 1 mm in the hepatic duct^[53,54]. It is a frequent finding and occurs in 36%-57% of patients during follow up^[55]. It should always raise the suspicion of the presence of a CCA. The stricturing process may cause extrahepatic biliary obstruction leading to development of symptoms. Patient with jaundice, pruritis, right upper quadrant pain and abnormal biochemical studies have been deemed appropriate candidates for therapy. The goal of therapy is to relieve biliary obstruction.

The optimal non surgical management of these dominant strictures is still debatable. Endoscopic balloon dilatation both with and without stenting has been studied. Long term stent therapy (3 mo) has been shown to have a high rate (close to 50%) of complications of cholangitis/jaundice attributed to stent occlusion^[56]. However, short term stenting (11 d) was associated with a lower rate (7%) of these complications, at the same time producing significant effects in symptom reduction and biochemical resolution of cholestasis. Additionally, 81% remained asymptomatic over a 19 mo follow up period and none of the patients had recurrence of clinical/biochemical cholestasis^[57]. Another study reported similar positive results after short term stent therapy with significant effects on resolution of symptoms and biochemical cholestasis with 80% of patients remaining re-intervention free at the end of 1 year^[58]. A randomized trial comparing balloon

dilatation and stenting has not been performed. However, a retrospective study that compared at endoscopic dilatation with dilatation + stenting reported an increased number of complications and incidence of cholangitis in the group that received both without significant difference in improvement of cholestasis^[59]. It has been suggested that the group that received both interventions may have been sicker as stenting was done only when dilatation alone did not improve biliary drainage^[60]. Moreover, half of the stents were placed percutaneously and the authors reported a significantly higher rate of complications with percutaneous placement of stents as compared to the endoscopic approach. One study aimed at assessing a survival benefit of endoscopic treatment of strictures reported a 5 years survival that was significantly ($P = 0.027$) higher over that predicted by the Mayo risk score^[61]. Indirect evidence from other studies has supported this finding^[62,63].

Overall, endoscopic therapy of strictures has been proven to be a safe and efficacious mode of treating primary sclerosing cholangitis associated strictures that helps in amelioration of symptoms and cholestasis with a low rate of complications. If stenting is considered, short term therapy is deemed best.

Surveillance for cholangiocarcinoma

Patients with PSC are at risk of developing CCA. The risk after 10 years and 20 years is 9% and 19% respectively^[64]. Patients with deterioration in functional status, worsening liver functions and/or weight loss should be evaluated for CCA. The distinction between a benign dominant stricture and CCA, however, has remained a challenge.

A cut off value of CA19-9 (cancer antigen 19-9) of > 130 U/mL in symptomatic patients has a sensitivity and specificity of 79% and 98% respectively^[65]. Its value as a screening tool in asymptomatic patients remains to be defined. CCA often presents in its advanced stage when identification of a mass lesion makes a diagnosis of CCA very likely. In early stages, however, diagnosis is difficult. A study that followed 230 patients over 6 years reported sensitivity of ultrasound, CT, MRI as 57%, 75% and 63% respectively when imaging alone was considered. The positive predictive value of ERCP, MRCP and MRCP + MRI was 23%, 21% and 23 % respectively^[66].

Bile duct brushings are the most commonly used method for tissue sampling during ERCP are now routinely obtained at the time of ERCP^[7-9]. The vast majority of extrahepatic CCA are periductal, cancers, and do not demonstrate mass lesions on imaging studies. Brush cytology obtained during ERCP is the usual diagnostic technique in the investigation of patients with biliary strictures. However the sensitivity of brushings is low for distinguishing benign strictures and CCA with a 43% sensitivity and a diagnostic accuracy of 50%-60%^[7-9].

At the time of ERCP brushings of stricture for cytology, another set of brushings for FISH are obtained in our institution. FISH probes are used to target the centromeric regions of chromosomes 3, 7 and 17 and the

9p21 band (p16). Some studies have considered positive FISH based on polysomy only, while some have considered trisomy or tetrasomy as positive too. In a recent meta-analysis from our group, we pooled all the available evidence in order to better define the utility of FISH for detection of CCA. Our unpublished observations show that any FISH positivity has a pooled sensitivity and specificity of 68% (95%CI: 61%-74%) and 70% (95%CI: 66%-73%) respectively. Thus both brushings for cytology and FISH obtained at the time of ERCP contribute significantly in diagnosing CCA in PSC patients^[9].

Recent studies have demonstrated promising results of cholangioscopy in the diagnosis of CCA. Tischendorf *et al.*^[67] prospectively studied 53 patients out of which 12 patients were found to have CCA based on tissue sampling. Patients underwent cholangioscopy in addition to ERCP, cholangioscopy was found to have higher sensitivity and significantly higher specificity, positive predictive value and negative predictive value than endoscopic retrograde cholangiography. A single center prospective study involving 36 patients and using peroral cholangioscopy and biopsy reported an overall accuracy of 89% in differentiating benign from malignant stenoses^[68].

ERCP with probe-based confocal laser endomicroscopy is an emerging technology that enables high resolution assessment of gastrointestinal mucosal histology thereby allowing examination of "optical biopsies" and exponentially expanding the scope of imaging capabilities. The examination is done *in vivo* and images are displayed in real time. A single center small case series of 15 patients and 21 dominant stenoses reported 100% sensitivity and 100% negative predictive value in excluding neoplasia^[69]. It concluded that if verified in large prospective studies, this technology could be used to risk stratify strictures in patients with PSC. Also, chromoendoscopy using methylene blue was studied for the first time in staining tissue of the bile duct^[70]. It helped identify normal, dysplastic and inflamed mucosa of the biliary tract as was subsequently proven by follow up or histology. A homogenous staining was suggestive normal tissue, absence of it on circumscribed lesions or diffuse staining predicted neoplastic or inflamed tissue^[70].

We routinely send 2 sets of brushes with PSC-related dominant strictures; one for routine cytology and the other for FISH analysis. Thus an advanced endoscopist forms an integral part of the team managing IBD patients with associated PSC and its related complications and surveillance of these patients for CCA.

IBD and colorectal neoplasia surveillance

Patients with IBD are at increased risk of CRC. Incidence of cancer has been more extensively studied in relation to UC than Crohn's. The risk in UC is increased with the duration and extent of disease. A meta-analysis reported the risk is 2% at 10 years, 8% at 20 years, and 18% at 30 years of disease^[71]. Patients with extensive colitis are at increased risk as compared to those with left sided colitis. Compared to age-matched controls, the risk begins to increase about 8-10 years after onset of symptoms. Patients

with PSC are also more predisposed to developing CRC. Earlier studies on the risk of CRC with CD have been inconclusive. It is now believed that risk of developing CRC in UC and CD is nearly identical^[72].

There have not been randomized controlled trials proving the effectiveness of surveillance colonoscopy. There have been three case series that have studied this^[73-75]. A Cochrane analysis of these studies concluded that there was no clear evidence that surveillance improves survival. There is evidence that cancer tends to be detected earlier in patients who undergo surveillance and they are likely to have a better prognosis although lead time bias may affect this apparent benefit. It stated that there may be indirect evidence that surveillance is effective at reducing death associated with IBD and that it may be acceptably cost-effective^[76].

Interval colonoscopies with random biopsies of abnormally appearing mucosa and targeted biopsies of suspicious lesions have been recommended. Newer methods aimed at detecting abnormal/dysplastic mucosa have been studied to help make this more effective. An advanced endoscopist with experience in complex mucosal imaging would play an important role in the surveillance of IBD patients for CRC. Newer endoscopic imaging modalities including high-definition endoscopy, chromoendoscopy, virtual chromoendoscopy and confocal laser endomicroscopy have the potential to significantly improve the detection and characterization of flat and subtle dysplasia, thereby setting the stage for a system of targeted neoplasia detection without random biopsies in IBD patients.

Chromoendoscopy is a dye-spraying technique that highlights the borders and surface architecture of neoplastic lesions, thereby unmasking and delineating subtle lesions and aiding in the differentiation of neoplastic and non-neoplastic tissue^[76]. Chromoendoscopy has been compared to standard-definition endoscopy for detection of neoplasia in both IBD and non-IBD patients and shown to be superior^[77]. However, careful cleansing and inspection of the entire colonic mucosal surface needs to be done to detect neoplasia^[76]. Importantly, the use of chromoendoscopy does not increase the mean procedure time and has been shown to be no better than white-light endoscopy with random and targeted biopsies^[78,79]. Chromoendoscopy described above using methylene blue has been studied in UC patients. The study included 102 patients, each of who had biopsies by different 3 techniques: standard colonoscopy with random biopsies, targeted biopsies and dye targeted biopsies in which methylene blue was segmentally applied throughout colon and abnormal mucosa that was made visible by dye spray was then biopsied. It concluded that dye targeted biopsies detected more dysplasia than random biopsies ($P = 0.001$) and more than targeted non dye biopsies. ($P = 0.057$)^[78]. The disadvantages of this technique include the cost, time consuming nature and the fact the dye does not always coat the mucosa evenly and does not allow for a detailed analysis of the subepithelial network.

Virtual chromoendoscopy technologies have also

been employed including narrowband imaging (NBI; Olympus, Tokyo, Japan), i-scan (Pentax, Tokyo, Japan), Fuji Intelligent Chromo Endoscopy (FICE; Fujinon, Tokyo, Japan) with use of selective light filters or post-image processing techniques to improve visualization of vascular structures^[80]. A recent randomized parallel group study with 112 patients randomized to get colonoscopy using either NBI or white lights reported no significant difference in detection of dysplasia^[81]. Another study involving 60 patients concluded that NBI was a less time consuming and equally efficacious method compared to chromoendoscopy to detect intraepithelial neoplasia in long standing IBD patients but had a high lesion and patient miss rate and cannot be recommended as a standard technique^[82].

Confocal laser endomicroscopy is another advanced imaging technique that permits direct histologic assessment of mucosa at the cellular and subcellular levels *in vivo*^[83]. The potential application of technology in IBD patients would be in combination with white-light endoscopy and chromoendoscopy to more accurately predict lesion histology and thus aid in decision making regarding lesion resectability in the setting of chronic colitis^[84].

Endoscopic mucosal resection (EMR) is being used for management of specific raised lesions in chronic UC patients getting surveillance colonoscopy for dysplasia. In a study, EMR was used to remove 79 flat Paris 0-II lesions with a recurrence rate of 2.4% at 3 mo with no additional lesions detected in a 4-year follow-up period^[85]. Thus management of dysplasia is emerging as a new intervention to develop in the area of IBD.

Even though considerable research has been done in the area of interventional IBD, future research is needed for better imaging techniques in the diagnosis of CCA and role of cholangioscopy in the management of PSC strictures. In addition, randomized controlled trials aimed at studying endoscopic balloon dilations and stenting in treating bowel strictures in the setting of IBD and comparing its efficacy with surgery are required.

The above review underlines the fundamental role of an advanced endoscopist in a team of IBD specialists, colorectal surgeons and hepatologists that manages patients with IBD. Further research and studies will better define the scope of the advanced endoscopist in the coming years.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

From conception to delivery: Managing the pregnant inflammatory bowel disease patient

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Core tip: Inflammatory bowel disease affects people during their reproductive years. Many patients and physicians have concerns about pregnancy in inflammatory bowel disease (IBD), and are unsure about management of IBD during pregnancy. Women with IBD have similar fertility as the general population, with the exception of certain prior surgeries, and active disease. This review highlights the relative safety of medications used to treat IBD during pregnancy and breastfeeding, and summarizes the updated literature for immunosuppressants and biologics. Good control of disease and clinical remission at the time of conception increases the likelihood of having successful pregnancy outcomes, and quiescent disease during pregnancy.

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Abstract

Inflammatory bowel disease (IBD) typically affects patients during their adolescent and young adult years. As these are the reproductive years, patients and physicians often have concerns regarding the interaction between IBD, medications and surgery used to treat IBD, and reproduction, pregnancy outcomes, and neonatal outcomes. Studies have shown a lack of knowledge among both patients and physicians regarding reproductive issues in IBD. As the literature is constantly expanding regarding these very issues, with this review, we provide a comprehensive, updated overview of the literature on the management of the IBD patient from conception to delivery, and provide action tips to help guide the clinician in the management of the IBD patient during pregnancy.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic bowel diseases, including Crohn's disease and ulcerative colitis, which can affect all aspects of a patient's life. The majority of patients with IBD are diagnosed in their adolescent to young adult years. The diagnosis of IBD and the medications and surgeries used to treat IBD come with many questions and concerns about how they can affect future education and work plans, and relationship and family plans. These questions and concerns not only affect the patient, but also the health care providers who are managing the patient. In particular, the complex

interaction between IBD and pregnancy is important, especially because any decisions regarding the management of the patient will not only affect her, but also her fetus. In addition, they will not only affect them currently, but may have long lasting effects.

The concerns regarding IBD and reproduction certainly play a central role in any decisions made by the patient and the physician caring for her. Many women with IBD choose not to become pregnant and to remain childless. This concept of “voluntary childlessness” has been documented in early studies that showed up to 30% of women with IBD (compared to 7% of the general population) had voluntary infertility^[1]. More recent studies confirm this “voluntary childlessness” continues to be an issue, with up to 18% of childless IBD patients indicating that the decision was influenced by IBD related factors^[2-4]. The five major concerns of women with IBD that have been reported by previous studies include: fertility/conception, genetics, IBD-related congenital abnormalities, medication effects on pregnant and fetus, and effect of pregnancy on IBD^[3-5]. Inadequate physician knowledge regarding reproduction in inflammatory bowel disease, and lack of comfort managing pregnant IBD patients may be contributing to the medical advice component of “voluntary childlessness” seen in IBD patients. In this review, we aim to address each of these major topics of concern, in the hopes that practitioners will be better equipped with up-to-date information to use in counseling their patients.

CONCEPTION

Fertility: what are the chances of becoming pregnant?

Women with IBD have been shown to have similar fertility as the general population^[1,2,6-9]. However, some studies report that Crohn's disease (CD) patients may have slightly decreased fertility^[10,11], especially when their disease is active^[9] or if they have adhesions from prior surgeries^[10,12]. Ulcerative colitis (UC) patients can have normal fertility, however once UC patients have had a surgery, such as restorative proctocolectomy and ileal pouch anal anastomosis, they have an increased risk of infertility up to 3-4 fold^[13-16]. It is hypothesized this increased infertility is due to tubal infertility from the adhesions and scarring^[15,16], since UC patients who have had laparoscopic IPAA have been shown to have less adhesions^[17], and lower infertility rates^[18,19]. Although there have been variable reports on the infertility rates among women with Crohn's disease and ulcerative colitis, some of these differences may be attributed to voluntary childlessness. In a recent meta-analysis of eleven studies, the authors found that in women with CD, fertility was reduced 17%-44% compared to controls, but further analysis revealed this to be linked to voluntary childlessness; they did not find any reduction in fertility in women with UC^[20].

Action point: In general, women with IBD have similar fertility rates as the general population. Previously reported infertility may be attributed to voluntary childlessness.

Women with Crohn's disease may have decreased fertility rates when their disease is active, or if have had prior surgeries. Women with ulcerative colitis who have had pelvic surgery have decreased fertility rates.

Genetics: what are the chances of offspring developing IBD?

Earlier studies supported strong genetic risks of IBD in offspring of patients with IBD up to 13 times the general population^[21,22]. When early twin studies were combined, results showed concordance ranging between 15.4% monozygote twin concordance for ulcerative colitis to 30.3% concordance for Crohn's disease^[23]. Monozygote twins are born from the same zygote, so this low concordance rate suggests there are other non-genetic influences, such as environmental factors. A recent study re-ran the Swedish twin registry, which is one of the major data sources for twin studies, and found that previous twin studies overestimated the influence of genetics in Crohn's disease^[24]. Although genetics does play an important role in the risk of developing IBD, they are not the only determinants.

Action point: Genetics play an important role in the risk of developing IBD, but women should be counseled that there are other factors involved. Their offspring will not necessarily develop IBD.

PREGNANCY

Conception and beyond: what is the effect of IBD activity on the pregnancy?

Some studies indicate that IBD, especially Crohn's disease, can be associated with an increase in adverse pregnancy outcomes, such as prematurity^[2,10,14,25-33], low birth weight^[14,25,26,28,31-32], small for gestational age^[25,28,30,33,34], congenital abnormalities^[28,31,35], miscarriages or spontaneous abortions^[11,30]. Other studies report no significant association between IBD and adverse pregnancy outcomes^[9,29,36-38]. However, the effect of IBD on pregnancy outcomes may be partially attributable to disease activity, and medications, rather than IBD alone. In addition, other demographic variables such as maternal age and smoking have been shown to be risk factors for congenital abnormalities and pregnancy outcomes such as preterm delivery, among women with IBD^[38].

A few studies concluded that disease activity did not predict adverse pregnancy outcomes in women with IBD^[1,30,32], but other studies found that active disease at the time of conception, and during pregnancy, increases the risk of adverse pregnancy outcomes, such as spontaneous abortion^[1,6,7] and preterm delivery^[37,39].

Action point: Women with IBD should be in remission before attempting to become pregnant.

Conception and beyond: what is the effect of pregnancy on IBD course?

Women with active disease at the time of conception, or

within 3 mo of conception, are more likely to have active disease during pregnancy than if their disease was in remission^[40,41]. For both ulcerative colitis and Crohn's disease, the relative risk of a women having active disease during pregnancy if she had active disease at the beginning of pregnancy is about two-fold^[41]. Conversely, women with disease in remission at the time of conception are more likely to have quiescent disease during pregnancy^[6]. Pregnancy was reported to decrease activity of Crohn's disease in a study of 61 women^[42], but a larger study including 92 women with Crohn's disease found no statistical significant difference in disease course during or after pregnancy^[43]. Longer disease duration in Crohn's disease patients may increase the risk of relapse during pregnancy^[43]. In ulcerative colitis patients, there was a higher risk of relapse during pregnancy and in the post partum period^[43]. A large study on 543 women over 10 years reported that pregnancy was associated with a reduced number of flares in the years following pregnancy^[44].

Action point: The disease activity at time of conception tends to predict disease course during pregnancy. Ideally, women should be in remission at the time of conception.

Flares and remissions during pregnancy: which medications can be used during pregnancy?

Women who require medications to achieve and maintain remission of their IBD, should continue these medications during pregnancy. With the exception of methotrexate, which should be stopped when attempting to conceive, and withheld during pregnancy, other medications used to manage IBD have not been associated with significant adverse fetal outcomes.

Aminosaliculates [Food and Drug Administration (FDA) Class B, Asacol FDA Class C] and sulfasalazine (FDA Class B): Aminosaliculates and sulfasalazine are commonly used drugs for mild to moderate ulcerative colitis. A cohort study from Denmark found an increased risk of stillbirth and preterm birth in women prescribed 5-ASA drugs during pregnancy, but was unable to distinguish between effects of disease activity and 5-ASA^[36]. Other studies found no significant association between 5-ASA drugs and poor pregnancy outcomes^[45-51]. A recent meta-analysis reported slight but non-significant increases in congenital malformation (OR = 1.16), stillbirth (OR = 2.38), spontaneous abortion (OR = 1.14), and preterm delivery (OR = 1.35) and low birth weight (OR = 0.93) with 5-ASA medications^[51]. However, again, the results were pooled, and they were unable to differentiate which 5-ASA drug, type of disease, or disease activity. Recently there has been concern about the dibutyl phthalate (DBP) coating on certain mesalamines, as animal studies found adverse effects on development and reproductive organs^[52]. A recent study reported high mean urinary concentrations of the main DBP metabolite in a woman who used Asacol^[52]. However, there have been no reports of adverse developmental or reproductive effects on hu-

mans. In addition, DBP can be found in many commonly used medications and dietary supplements^[53].

Sulfasalazine inhibits folate synthesis, so women on sulfasalazine should be supplemented with folic acid (5 mg/d) to prevent neural tube defects^[54,55]. Sulfasalazine can also displace bilirubin from albumin, which theoretically could lead to kernicterus in the newborn child, but no cases have been reported^[55].

Action point: Amniosaliculates and sulfasalazine can be used during pregnancy, and are not significantly associated with adverse neonatal outcomes. Women on sulfasalazine should receive folic acid supplementation (5 mg/d).

Azathioprine and 6-mercaptopurine/Purinethol (FDA Class D): Although thiopurines are classified as FDA Class D drugs, because of teratogenicities in animal studies, the use of azathioprine/6-MP during pregnancy in IBD is not associated with increased risk of preterm birth, low birth weight, neonatal adverse outcomes, or congenital abnormalities^[33,49,56-60]. Disease activity rather than medication use can lead to neonatal adverse outcomes^[57]. One study reported that thiopurines increased the risk for congenital malformations when compared to healthy women, but not when compared with IBD controls^[61]. A large ongoing prospective registry of pregnancy outcomes in women with IBD exposed to immunomodulators and biologic therapy (PIANO study) has found no association with the use of immunosuppressants with congenital anomalies, abnormal newborn growth and development, or other complications^[62]. In addition, a recent review found that thiopurine use during pregnancy was not associated with low birth weight or congenital abnormalities, but was associated with pre-term birth^[63]. Infants may be exposed to a metabolite of Azathioprine, 6-TGN^[64,65], and a recent study has found that up to 60% of infants exposed to thiopurines in utero are born with anemia^[65]. In long-term (average 4 years) follow-up studies of babies exposed to azathioprine in utero, there was no increased risk of infection^[66] or development and immune function^[67]. Expert opinion is to continue thiopurine use during pregnancy to maintain remission of disease^[54,68].

Action point: Thiopurines can be used during pregnancy, and are not significantly associated with adverse neonatal outcomes.

Methotrexate (FDA Class X): Methotrexate is a teratogen and an abortifacient, and is therefore contraindicated during conception and pregnancy period. Methotrexate exposure during organogenesis (6-8 wk) may lead to congenital abnormalities, while exposure in the second and third trimesters can lead to fetal loss^[55,69]. Since Methotrexate remains in the tissue for a period of time, patients should discontinue at least 3-6 mo prior to attempting to conceive^[54,69]. Women who become pregnant while on methotrexate should seek medical attention immediately, for assessment of the fetus, and counseling

regarding options^[54,55].

Action point: Methotrexate should be discontinued at least 3 to 6 mo before conception.

Corticosteroids (FDA Class C): Glucocorticoids cross the placenta and can reach the fetus, but the placental enzymes convert corticosteroids to less active metabolites^[54,55]. Prednisone, prednisolone, and methylprednisolone are the preferred agents during pregnancy, as they are more efficiently metabolized by the placenta than dexamethasone or betamethasone^[54]. Most studies on glucocorticoid use during pregnancy have been in patients with various diseases, such as asthma. There has been a reported association of increased oral cleft in neonates exposed to glucocorticoids in utero in the first trimester, and this risk should be discussed with the patient^[54,55,69]. Overall there is no increased risk of congenital abnormalities^[50]. Budesonide has only been reported in one small study in Crohn's disease patients, and was not associated with adverse neonatal outcomes^[70].

Action point: Corticosteroids may be used to treat flares of IBD during pregnancy. There is a small risk of oral cleft in neonates exposed to corticosteroids in the first trimester.

Antibiotics: Metronidazole (FDA Class B) and Ciprofloxacin (FDA Class C) are commonly used to treat abscesses and fistulae in IBD. Animal studies showed carcinogenic effects from Metronidazole, and early studies suggested a risk of cleft lip^[55], but this has not been reported in humans^[71]. It was not associated with preterm birth (OR = 1.02, 95%CI: 0.80-1.32), low birth weight (OR = 1.05, 95%CI: 0.77-1.43), OR = congenital anomalies (OR = 0.86, 95%CI: 0.30-2.45) in a large study of 2829 singleton/mother pairs^[72]. In a small study (27 of 113 patients on Metronidazole) in female IBD patients, metronidazole was found to be safe in all trimesters of pregnancy^[49]. Previously, there was concern that quinolones increase the risk of arthropathies in the offspring. Studies have reported no significant increase in major congenital anomalies, including musculoskeletal problems from the use of ciprofloxacin^[73]. A meta-analysis of pregnancy outcomes after exposure to quinolones in the first trimester reported no increased risk of major malformations, stillbirths, preterm births, or low birth weight^[74]. However, because of the known possible effect of ciprofloxacin on bone and cartilage, it has been recommended to avoid this medication during pregnancy^[55].

Penicillins have not been shown to cause fetal malformations or adverse pregnancy outcomes, and are considered the first line therapy in pregnancy^[71]. Amoxicillin (FDA Class B) can be used to treat abscesses and complications of IBD during pregnancy.

Action point: Metronidazole can be used during pregnancy, preferably avoid use in first trimester. Ciprofloxacin

should be avoided during pregnancy due to risk of arthropathy. Amoxicillin is safe to use during pregnancy.

Biologics: Anti-tumour necrosis factor inhibitors (FDA Class B) such as Infliximab, Adalimumab, and Certolizumab, are commonly used to treat moderate to severe IBD, and fistulizing Crohn's disease. TNF- α is a pro-inflammatory cytokine that stimulates the production of prostaglandins, and increased levels are associated with preterm labor^[75]. TNF levels increase during pregnancy, as it is mainly produced by the placenta^[76]. TNF- α is important for the initial stages of pregnancy, and also for the development of the fetal immune system, and TNF deficient animals have been shown to have increased risk of immune developmental abnormalities^[77]. However, increased levels of TNF- α have been associated with preeclampsia, gestational diabetes, obesity^[76].

Initially Infliximab and Adalimumab were reported in a few cases of pregnant women with IBD^[78-84] which did not show any adverse effects. Larger observational studies, registry studies, and systematic reviews have shown its safety for use during pregnancy^[85-88]. The PIANO study has not found any increase in congenital anomalies, abnormal newborn growth and development, or other complications, among women receiving biologics^[62].

Infliximab and Adalimumab are IgG1 monoclonal antibodies, and are actively transported across the placenta, while Certolizumab is a Fab fragment of IgG1, and has not been shown to have placental transportation^[89]. This active transport of IgG1 antibodies occurs mainly in the third trimester^[81,90]. Thus it has been recommended to stop Infliximab and Adalimumab at the onset of the third trimester^[91,92]. However, in a recent study, in women with quiescent IBD, who discontinued anti-TNF therapy by week 30, Infliximab and Adalimumab were still detected in cord blood^[93]. The exact time to hold anti-TNF is now debatable, especially with Adalimumab which is given weekly or biweekly, but in high risk patients, or patients with active disease, these biologics should be continued throughout the pregnancy^[94]. Levels of Infliximab and Adalimumab have been detected in infants for as long as 6 mo^[95]. At least for the short term, children exposed to intra-uterine Infliximab develop normally, without increased infections, allergic reactions, or decreased response to vaccinations^[91]. However, infants exposed to combination of immunomodulators and biologics have been noted to have increase in infections from 9 to 12 mo of age^[62]. Thus, it is still recommended that infants exposed to intra-uterine anti-TNF therapy delay live vaccinations for at least the first 6 mo.

Action point: Anti-TNF therapies are safe to use during pregnancy. Infliximab and Adalimumab should be held after week 30, if not earlier, to decrease placental transport to the fetus. Neonates exposed to biologics during pregnancy should not have live vaccines during the first 6 mo post delivery.

Cyclosporine (FDA Class C): Cyclosporine crosses the placenta, and has not been found to be teratogenic in animal models^[96]. The majority of studies on cyclosporine in pregnancy involve post-transplant patients, which suggests an association with premature delivery and low birth weight infants^[97]. In severe ulcerative colitis flares during pregnancy, cyclosporine has been used with successful control of the disease, avoidance of colectomy during pregnancy, and no significant adverse pregnancy outcome^[98-104]. The most common side effect reported was hypertrichosis in the mother, however, in one case report, the patient developed severe hypertension and seizures 48 h post infusion^[99]. Other adverse effects of cyclosporine include nephrotoxicity and hepatotoxicity^[97].

Fulminant ulcerative colitis leading to colectomy has been associated with adverse pregnancy outcomes, with up to 49% fetal mortality and 22% maternal mortality rates in the literature^[105]. Thus, in cases of severe fulminant ulcerative colitis, in order to avoid urgent colectomy, cyclosporine may be considered.

Action point: Cyclosporine may be considered in cases of severe fulminant ulcerative colitis in pregnancy, in order to avoid colectomy during pregnancy. However, as biologics are FDA Class B, and there are more studies on Infliximab use during pregnancy, Infliximab may be the preferred first line option.

Managing relapses during pregnancy: can we use induction medications?

As already mentioned, active disease during pregnancy is associated with poor pregnancy outcomes. Since medications commonly used to treat IBD are not associated with significant adverse pregnancy outcomes, treating the mother to induce and maintain remission during the remainder of the pregnancy will lead to more beneficial outcomes. If hospitalization is required to manage an acute IBD flare, IV hydrocortisone^[103] and IV infliximab^[78,82,91,92,106] may be used for rescue therapy, as thus far, they have not been associated with significant adverse effects. One study has shown that IV cyclosporine can be used safely^[103].

Action point: Active IBD is associated with adverse pregnancy outcomes. Management of flares of IBD during pregnancy may involve the use of steroids, biologics, and possibly cyclosporine.

DELIVERY

Women with IBD were initially reported to be more likely to have a caesarean section^[2,14,25,33,34,107]. This has mainly been attributed to Crohn's disease patients, especially those with perianal disease^[14,107,108]; it has also been shown that vaginal delivery with episiotomy may be associated with subsequent perianal involvement^[109]. Larger population studies found no significant difference in caesarean section rates among IBD patients^[38], and no

risk of progression of perianal disease in Crohn's disease with vaginal delivery^[108]. Thus, the decision for caesarean section should not be made purely on the IBD diagnosis, but also obstetrical reasons.

Action point: In women with Crohn's disease with active perianal disease, caesarean section should be considered on an individual basis.

POST PARTUM

What is the risk of IBD flaring after delivery?

Some women may flare after delivery, while others fare well. A retrospective cohort study of 114 Crohn's disease patients reported more frequent disease progression after childbirth in patients who had active luminal disease prior to pregnancy^[108]. A large multi-country prospective study found higher risk of relapse in the postpartum period in women with ulcerative colitis^[43].

Action point: Ulcerative colitis patients have an increased risk of relapse after delivery. Crohn's disease patients with active luminal disease before pregnancy have a higher risk of relapse after delivery.

BREASTFEEDING

Breastfeeding physiologically may be associated with increased inflammation, as prolactin is associated with up-regulation of TNF production^[110] and increased levels are found in other autoimmune diseases such as lupus, rheumatoid arthritis^[111]. Many women with IBD choose not to breastfeed their children^[2,112]. This may be due to fears of medication effects, physician recommendation or personal choice^[112]. The effect of breastfeeding on the development of IBD is thought to be related to the hygiene hypothesis, in which breastfeeding is thought to help the neonate develop tolerance to microflora and food antigens, thus preventing immune over activation to delayed antigen exposures^[113-115].

Does breastfeeding affect IBD?

Studies investigating the effect of breastfeeding on developing IBD vary in methodology and conclusions. In one study, women who breastfeed were found to be more likely to have a postpartum flare of their disease, but this increased risk was non significant once adjusted for discontinuation of IBD medications during pregnancy^[112]. A more recent population-based study found no increased rate of disease flare in the post partum year between those who breastfed (26%) *vs* those who did not (29.4%)^[116].

Can breastfeeding affect the risk of IBD in the offspring?

Some studies find no association between breast feeding and diagnosis of IBD^[117]. However, some report that a lack of breastfeeding in infancy is associated with an increased risk of UC (OR = 1.5, 95%CI: 1.1-2.1) and

CD (OR = 1.9, 95%CI: 1.1-3.3)^[118]. More recent studies reported a protective effect of breastfeeding to decrease the odds of developing IBD^[119,120]. Two systematic reviews investigating the role of breastfeeding and the development of IBD found that breastfeeding is associated with lower risks of developing early-onset IBD^[121,122].

Which medications can be used during breastfeeding?

Aminosalicylates (FDA Class B, Asacol FDA Class C) and sulfasalazine (FDA Class B): Earlier studies have reported the rare occasion of infants exposed to 5-aminosalicylates via breast-milk developing watery diarrhea^[123], however very small amounts of drug are excreted into breast milk, making risk of toxicity and reaction very unlikely^[124-126]. Sulphasalazine has also been reported to cause bloody diarrhea in the infant exposed via breast-milk^[127], however, it has not been reported other than case reports. Sulfasalazine can have a bilirubin displacing effect leading to jaundice in the neonate, however the amount of drug transferred to the child via breast-milk is negligible to cause jaundice^[128,129].

ACTION POINT: 5-aminosalicylates and sulphasalazine can be continued during breastfeeding.

Azathioprine and 6-mercaptopurine/Purinethol (FDA Class D): Azathioprine and 6-MP can be continued during pregnancy, as described above, but there are concerns about the potential for tumorigenicity, and increased susceptibility to infections in neonates exposed during breastfeeding^[66]. Recent studies show that only very small amounts of AZA/6-MP are measured in breast milk, and negligible amounts detected in the neonate^[130-134]. In addition, the highest concentration of AZA measured in the breast milk appears during the first 4 h after consumption^[130], thus it has been recommended to “pump and dump” the first 4 h of breastmilk. Thus far, there has not been reported increase risk of infections among babies breastfed with exposure to azathioprine^[66], and it is considered safe to continue these medications during breastfeeding^[54,66,130-134].

Action point: Azathioprine and 6-MP can be continued during breastfeeding; the first 4 h of breastmilk after consumption may be discarded to minimize the amount of drug transferred to the neonate.

Methotrexate (FDA Class X): Methotrexate crosses into the breast milk^[135] and because of its teratogenicity, it is contraindicated during breastfeeding^[136,137].

Action point: Methotrexate is contraindicated during breastfeeding.

Corticosteroids (FDA Class C): Corticosteroids do transfer to the breast milk, but in very low levels^[138,139] and because the highest levels appear in the first 4 h^[139], it is recommended to “pump and dump”^[55,139] the first 4

h after medication consumption to minimize transfer of the drug to the neonate.

Action point: Corticosteroids can be continued during breastfeeding if required to treat maternal IBD.

Antibiotics: Metronidazole is transferred into the breast milk^[139], but in minimal levels^[140] and levels decline after 12-24 h after maternal dose intake^[141]. If metronidazole is required for the treatment of active IBD, it is recommended to wait 12-24 h after metronidazole intake before breastfeeding^[55], and long term use should be avoided^[69]. Ciprofloxacin is also detectable in the breast milk in small amounts^[142,143], but short term treatment can be used if indicated^[55].

Action point: Metronidazole and ciprofloxacin can be continued for short term during breastfeeding if required to treat maternal IBD.

Biologics: As mentioned, the biologic therapies can be continued during pregnancy, and held in the third trimester. Studies have shown nil to minimal levels of infliximab and adalimumab in the breast milk and no significant adverse events have been reported in the infant^[81,143-148]. It is thought that any detectable levels in the neonate after delivery may be due to placental transfer during pregnancy^[147]. Thus, although anti-TNF therapies can be continued during breastfeeding, further studies are required to determine the effect of infant exposure to these biological therapies on the development of their gastrointestinal immunity and systemic immune system^[89,146,148]. The preliminary results of the PIANO study have not found any association between breastfeeding and infection risk in the neonate exposed to biologic therapy^[62].

Action point: Infliximab and adalimumab may be continued during breastfeeding.

Cyclosporine (FDA Class C): Cyclosporine does cross into the breast milk, but if required for fulminant colitis, it can be used. Case reports and series of neonates exposed to cyclosporine during pregnancy and breastfeeding are mainly from the renal transplant literature, and have reported varying levels of cyclosporine in the breast milk, and relatively good outcomes in the mother and neonate^[149-153]. One case report of cyclosporine use in the management of severe ulcerative colitis while breastfeeding also reported short term good outcomes in the mother and neonate^[154]. More studies are required to determine the long term effects of neonatal exposure to cyclosporine in the breast milk.

Action point: Cyclosporine has been used to manage fulminant colitis during breastfeeding, however, infliximab is preferred due to the lack of studies for cyclosporine.

CONCLUSION

IBD affects people during an important time of their lives when they are considering family planning or are already pregnant. With the exception of Methotrexate, commonly used medications for the treatment of IBD are not associated with significant adverse pregnancy outcomes, and can be used throughout pregnancy. Good control of disease and clinical remission at the time of conception increases the likelihood of having successful pregnancy outcomes, and quiescent disease during pregnancy. There is no need to adjust medications during pregnancy, with the exception of biologics such as Infliximab and Adalimumab, which should be held during the third trimester in women who are in clinical remission. However, biologics may be continued throughout pregnancy if necessary to control disease. Induction of remission of IBD flares during pregnancy should be treated with appropriate medications, such as steroids, infliximab, and for severe fulminant colitis, cyclosporine, as active disease and fulminant colitis requiring surgery has increased risk of adverse fetal outcomes. The management of IBD in women during their reproductive years should include consideration of their family planning decisions, and education counseling regarding the overall safety of medications and the importance of medication adherence should occur prior to conception.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Glucose intolerance and diabetes mellitus in ulcerative colitis: Pathogenetic and therapeutic implications

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Abstract

Diabetes mellitus is one of the most frequent co-morbidities of ulcerative colitis patients. The epidemiological association of these diseases suggested a genetic sharing and has challenged gene identification. Diabetes co-morbidity in ulcerative colitis has also relevant clinical and therapeutic implications, with potential clinical impact on the follow up and outcome of patients. These diseases share specific complications, such as neuropathy, hepatic steatosis, osteoporosis and venous thrombosis. It is still unknown whether the coexistence of these diseases may increase their occurrence. Diabetes and hyperglycaemia represent relevant risk factors for postoperative complications and pouch failure in ulcerative colitis. Medical treatment of ulcerative colitis in patients with diabetes mellitus may be particularly challenging. Corticosteroids are the treatment of choice of active ulcerative colitis. Their use may be associated with the onset of glucose intolerance and diabetes, with difficult control of glucose levels and

with complications in diabetic patients. Epidemiologic and genetic evidences about diabetes co-morbidity in ulcerative colitis patients and shared complications and treatment of patients with these diseases have been discussed in the present review.

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Key words: Diabetes mellitus; Ulcerative colitis; Diabetes complications; Inflammatory bowel diseases; Glucose intolerance; Medical therapy; Corticosteroids

Core tip: The relationship between ulcerative colitis and diabetes mellitus is intriguing, full of practical and speculative information, useful for clinical practice and basic research. Diabetes mellitus is one of the most frequent co-morbidities of ulcerative colitis and their epidemiological association suggests genetic sharing and stimulates studies for gene identification. Diabetes also shares specific complications with ulcerative colitis and represents a challenging condition in ulcerative colitis patients for the treatment of the disease, due to difficult control of glucose levels and for high risk of postoperative complications and pouch failure. All these issues have been discussed in the present review.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic disease characterised by mucosal inflammation, limited to the colon. Its inci-

dence and prevalence are increasing with time in different regions around the world, with the highest annual incidence of 24.3 per 100000 persons-year, and prevalence of 505 per 100000 persons in Europe^[1].

The disease is associated with high costs, disease-specific morbidity and decreased quality of life, mostly related to complications, surgery and co-morbid diseases.

Co-morbid diseases in UC include several immune mediated diseases, such as rheumatoid arthritis, multiple sclerosis, lupus, psoriasis, hypothyroidism and diabetes mellitus^[2-7].

Among these diseases, diabetes mellitus is the most frequent condition and its association with UC has epidemiological, pathogenetic, clinical and therapeutic implications.

All these points represent the objects of the present review.

An electronic literature search was conducted using PubMed and Medline as primary sources. No time limits were specified up to the date of the search (September 2013). A comprehensive search was performed using the following search terms: “ulcerative colitis” or “inflammatory bowel diseases” and “diabetes mellitus” or “glucose intolerance”. The search was restricted to articles involving humans and those in the English language (or with an English abstract) and following identification of relevant titles, the abstracts of these articles were read to decide if the study was eligible.

The full text article was retrieved when the title and/or abstract seemed to meet the pre-defined eligibility criteria. A manual cross-reference search of bibliographies was carried out to identify articles missed in the computerised search.

DIABETES CO-MORBIDITY IN UC

Diabetes mellitus, like other autoimmune disorders, is significantly associated with UC, both in children and in adult patients. A recent large case-control study, which included more than 1200 children with inflammatory bowel disease (IBD), 488 of whom with UC, showed that UC is associated with a higher prevalence of diabetes than in controls (OR = 2.7, 95%CI: 1.1-6.6) with an overall prevalence of 2049 cases per 100000 children. Noteworthy, this association was confirmed excluding patient on treatment with anti-TNF alpha, and was the strongest among the other autoimmune conditions. It was also specific for UC, the association with Crohn's disease not being significant (OR = 1.4, 95%CI: 0.5-4)^[7]. Regarding the association between UC and diabetes, the estimates in this paediatric study were only slightly higher than those in adult studies. Indeed, few other studies examined the concomitance of autoimmune diseases among adult IBD patients without showing a significant increased risk for type 1 diabetes mellitus^[3,4] although this endocrine disorder represented the third most common co-morbid disease in UC patients (0.8%) after psoriasis (1.8%) and rheumatoid arthritis (1.1%). No study

has assessed the prevalence of type 2 diabetes mellitus in IBD, so far.

Interestingly, patients with both psoriasis and IBD showed significantly higher rates of diabetes (26.7% *vs* 11.0%) and autoimmune thyroiditis (2.1% *vs* 6.8%) and hepatitis (0.7% *vs* 6.2%) compared to individuals with psoriasis only^[8].

On the other hand, a large study made possible by the availability of the Multigeneration Register in Sweden, estimated the associations between type 1 diabetes mellitus and 33 autoimmune and related diseases in parents, offspring, siblings and twins. This study showed that type 1 diabetes in offspring was associated with 13 diseases in parents, including UC (standardised incidence ratio 1.23) and few other gastrointestinal diseases such as primary biliary cirrhosis (3.63) and celiac disease (2.73)^[9].

All these epidemiological findings suggest potential shared aetiological mechanisms for UC and type 1 diabetes mellitus. Although the aetiology for both diseases remains unclear, a large body of evidence supports the hypothesis that in genetically predisposed individuals, both host factors and environmental factors contribute to an uncontrolled immune function.

Co-morbidity among these autoimmune disorders and familial associations with several autoimmune and related diseases suggest genetic sharing and represent a challenge for gene identification.

On this regard, a recent genome-wide association study examined known susceptibility loci for IBD and type-1 diabetes mellitus in a cohort of 1689 Crohn's disease patients, 777 UC patients, 989 type-1 diabetes patients and 6197 control subjects, and identified multiple shared loci with opposite effects. In particular the study identified 1 diabetes mellitus locus (TNFAIP3) that confers UC risk and 2 UC loci (HERC2 and IL26) that confer type-1 diabetes mellitus risk^[10].

The genetic association between UC and type-1 diabetes mellitus has been also suggested by the description of a monogenic form of diabetes with the typical features of type 1 diabetes (autoantibodies to β cells, lean and young at onset of hyperglycemia, rapid disappearance of C-peptide production and insulin dependence) together with insulin resistance, which appears as a consequence of an autosomal-dominant mutation in the *SIRT1* gene. A recent case-report describes a family carrying a mutation in the *SIRT1* gene, in which all five affected members developed an autoimmune disorder: four members developed type 1 diabetes and one developed UC^[11]. It is particularly interesting to know that SIRT1 suppresses TNF α expression^[12]. Importantly, both type-1 diabetes and UC are strongly associated with this cytokine, and TNF antagonism improves both conditions^[13].

COMMON SHARED COMPLICATIONS IN DIABETES AND UC

Diabetes mellitus and UC share a number of complications, namely neurological, hepatobiliary, osteoarticular,

Table 1 Common shared complications in diabetes mellitus and ulcerative colitis

Neurological
Distal symmetric polyneuropathy (50% in DM and 0%-39% in UC)
Hepatobiliary
Cholelithiasis (20%-30% in DM, only after colectomy in UC)
Hepatic steatosis
Non alcoholic fatty liver disease
Osteo-articular complications
Osteoporosis
Vascular complications
Venous thrombosis (with ketoacidosis in DM, with active disease or surgery in UC)
Post-operative complications
Anastomotic dehiscence
Infections
Non-infectious complications

DM: Diabetes mellitus; UC: Ulcerative colitis.

vascular and post-operative. It is unknown whether concomitance of both diseases for a long time increases the risk of such complications.

Although diabetic patients with mild or quiescent UC are likely to have a favourable outcome, UC patients with recurrent or steroid-refractory active disease, with consequent long term steroid treatment, could present hyperglycaemia and hyperinsulinemia and an increased risk of complications. Unfortunately, the outcome of UC in diabetic patients has not been investigated so far and no data are reported in the large therapeutic trials.

Examples of the more frequently shared complications of diabetes mellitus and UC are presented hereafter (Table 1).

Neurological complications

Neuropathy is a well known complication of diabetes mellitus. In particular, distal symmetric polyneuropathy is the most frequent form of neurological involvement, occurring in up to 50% of diabetic patients^[14]. Peripheral neuropathy is also a neurological complication of inflammatory bowel diseases^[15,16] with an incidence ranging from 0% to 39%, depending on the features of the study populations and on the criteria adopted to define the neuropathy. A population-based study showed that incident at peripheral neuropathy in IBD patients occurs late in the course of the disease^[17] and is likely due to nutritional (*e.g.*, B₁₂ deficiency), iatrogenic (*e.g.*, metronidazole neurotoxicity) and immune-mediated causes.

Hepatobiliary complications

Cholelithiasis, a well known complication of 20%-30% of patients with diabetes mellitus probably due to impaired gallbladder contraction, obesity and hyperlipidemia^[18], is also reported as a complication in UC patients, but only after colectomy, likely due to changes in bile composition and increase in cholesterol concentration in bile^[19].

Hepatic steatosis is a frequent feature of both diabetes mellitus and UC. Nonalcoholic fatty liver disease

(NAFLD) is characterized by insulin resistance and it is often associated with type 2 diabetes mellitus^[20,21]. Up to 50% of these patients may have nonalcoholic steatohepatitis^[22]. NAFLD is also frequently reported in UC patients, apart from classical risk factors such as obesity or insulin resistance^[23].

Osteo-articular complications

Diabetes mellitus-induced osteoporosis is often present in diabetic patients, probably due to changes in osteoblast function and bone formation, sustained by hyperglycemia^[24].

A high prevalence of reduced bone density is also frequently reported in UC patients due to increased bone reabsorption, not balanced by an appropriate bone formation. Long duration of disease, low body mass index, colectomy and in particular high doses and prolonged treatment with corticosteroids (cumulative use of steroids) have been recognised as risk factors for this complication and fractures^[25-27].

Vascular complications

Diabetes mellitus and UC share vascular complications, such as venous thrombosis. UC is characterised by a potential hypercoagulable state and an incidence of systemic thromboembolic events higher than in the general population, usually correlated with active disease and surgery^[28-30]. Diabetes, although most commonly complicated by arterial thrombosis, may also be complicated by venous thromboembolism, in presence of ketoacidosis^[31-33].

Post-operative complications

Diabetes mellitus is a well known risk factor of poor outcome in colorectal surgery, mainly due to the occurrence of anastomotic dehiscence^[34], infectious and non-infectious complications.

Recently, it has been found that perioperative hyperglycemia, in diabetic patients and even in patients without a preoperative diagnosis of diabetes undergoing colorectal surgery, is associated with a high rate of infectious and noninfectious complications, reintervention and mortality^[34].

Adverse outcomes may be associated with a single postoperative elevated glucose value and the risk of morbidity and mortality is related to the degree of hyperglycaemia^[35,36].

Therefore, it is not surprising that the post surgical period is one of the most important time frames for morbidity and mortality in UC patients with co-morbid diabetes mellitus.

Surgical-site infections is a major source of morbidity after colectomy for fulminant UC. A retrospective study including 59 patients operated for fulminant UC, showed that diabetes is one of the most frequent independent risk factors for surgical-site infections, along with white blood cell count, intraoperative blood loss and blood transfusion^[37]. The poor outcome of the postopera-

Table 2 Predictive risk factors for the development of diabetes mellitus and hyperglycemia in ulcerative colitis patients treated with corticosteroids

High dose of corticosteroids
Long duration of corticosteroids therapy
Advanced age
High body mass index
Family history of diabetes
Previous gestational diabetes

tive course in diabetic patients with UC has been also found in another large retrospective study that included 3754 patients undergoing ileoanal pouch, which showed that diabetes mellitus was an independent factor associated with the risk of pouch failure (HR = 2.31; 95%CI: 1.25-4.24)^[38].

CORTICOSTEROID-INDUCED DIABETES IN UC

IBD are immune-mediated disorders which appear in genetically predisposed subjects.

Corticosteroids are the main therapeutic agents in UC, because of multiple effects on the cellular and humoral immune system, including an inhibitory action on several pro-inflammatory cytokines and metabolites of arachidonic acid.

For more than 50 years corticosteroids, such as prednisone and methyl-prednisolone, have been used to treat IBD during the acute phase. However, more than 50% of patients do not respond to the therapy (steroid-resistance) or have a relapse after treatment discontinuation (steroid-dependence) and about half of them show side effects of variable severity^[39,40]. In most cases, the appearance and the seriousness of side effects (except from osteonecrosis and idiosyncratic reactions) are related to duration and dose of therapy.

Hyperglycemia and corticosteroid-induced diabetes are the most common systemic manifestations in IBD under steroid treatment and represent a real problem in the handling of UC patients with diabetes mellitus when relapses of the intestinal disease occur.

To date, there are few data on the incidence of corticosteroid-induced hyperglycemia or diabetes in IBD and also about the onset of acute diabetic complications, such as ketoacidosis and hyperosmolar hyperglycemic state, in diabetic patient affected by IBD, under steroid treatment. Most of our knowledge is derived from non-gastroenterological studies.

A case-control study, conducted on 55 elderly patients with active Crohn's disease compared to 66 control subjects not treated with steroids, showed that treatment with high doses of corticosteroids may increase the risk of hyperglycemia, even if the difference was not statistically significant (RR = 1.53; 95%CI: 0.54 - 4.32)^[41].

However, another case-control study that included a large number of patients (11855 cases and 11855 controls) showed that corticosteroids (prednisone or ana-

logues at a dose of 30 mg a day or more) confer a RR = 10 of hyperglycemia, compared to non treated patients^[42].

A retrospective study, including 25 patients affected by neuropathy (median age: 50 years) showed that treatment with prednisolone at a dose of 30-60 mg a day for at least 2 wk may result in a postprandial hyperglycemia, compatible with diabetes mellitus in 13 of 25 patients and demonstrated that advanced age is a risk factor for this complication^[43].

The importance of age in the onset of corticosteroid-induced diabetes is confirmed also by a cohort study conducted on a large geriatric population (median age: 75 years) that shows an increased risk of diabetes induced by oral corticosteroids (RR = 2.31; 95%CI: 2.11-2.54), compared to treatment with proton-pumps inhibitors (PPIs)^[44].

Finally, a recent literature review has confirmed that corticosteroid-induced hyperglycemia is common both in patients with and without diabetes and it has estimated an OR = 1.5-2.5 for the onset of diabetes mellitus in treated patients^[45].

Overall, the available studies, mostly conducted on patients not affected by IBD, confirm that the total dose of corticosteroids and the long duration therapy are important predictive risk factors for the development of diabetes mellitus. In addition, these studies underline that other factors, such as advanced age, high BMI, family history of diabetes or previous gestational diabetes should be considered and recommend monitoring the blood glucose level during steroid therapy (Table 2).

The possible underestimation of this condition in the handling of patient with IBD in clinical practice could be attributed to the short duration of steroids treatment and to the importance given to the fasting blood glucose only. High blood glucose levels in the short-term and in the postprandial period, should be considered for their prognostic value.

Concerning the role of the corticosteroids dose on the onset of diabetes, high doses of steroids are associated with high values of blood glucose levels and can induce diabetic ketoacidosis in patients with type 1 diabetes mellitus or hyperosmolar hyperglycemic state^[46,47].

The risk of these complications is particularly significant in diabetic patients with UC, in whom the first-line treatment is represented by corticosteroids.

TREATMENT OF UC IN DIABETIC PATIENTS

Treatment of patient with UC depends mainly on the activity and location of disease^[48,49].

Severe UC

Severe UC is characterized by bloody diarrhoea > 6 bowel movements/d and many signs of systemic toxicity (tachycardia > 90 bpm, fever > 37.8 °C, Hb < 10.5 G/dL or ESR > 30 mm/h)^[50].

Patients with severe UC should be admitted to hospi-

Table 3 Management of active severe ulcerative colitis in diabetic patients treated with corticosteroids

Disease monitoring	Disease treatment
Regular monitoring of blood glucose level	Rehydration with saline solution
Regular monitoring of disease activity (e.g., Disease Activity Index)	Correction of blood glucose levels
Plain abdominal X-ray	Treatment of hypokaliemia
Dosage of:	Treatment of hypomagnesaemia
C-reactive protein	Consider alternative treatments
Blood cell count	<i>Iv</i> cyclosporine A (4 mg/d)
Electrolytes	Infliximab (5 mg/kg or 10 mg/kg)
Anion gap	Adalimumab (160 mg/80 mg/40 mg eow)
Osmolality	Tacrolimus
Serum creatinine levels	Leucocyapheresis
Ketones	Other therapies (vedolizumab, visilizumab, abatacept, tofacitinib)
Urinalysis	
Blood gas analysis	

tal for intensive treatment^[49].

Corticosteroids, administered parenterally (e.g., Methylprednisolone 60 mg daily or hydrocortisone 100 mg four times daily), represent the first-line treatment of severe UC. Higher doses are not more effective, but lower doses are less effective^[51]. Duration of treatment is 7-10 d, since further extension of therapy carries no additional benefit. Response to therapy reaches 67% and non-responders UC patients require a second-line treatment, represented by cyclosporine, tacrolimus, infliximab or colectomy^[52].

Steroid treatment of severe acute UC, in particular in diabetic patients, requires particular attention to hyperglycemia induced by therapy. The combination of dehydration, electrolyte imbalance (hypokalemia and hypomagnesemia), the possible presence of a septic condition and the need of total parenteral nutrition are important risk factors for hyperosmolar hyperglycemic state and diabetic ketoacidosis, the major complications of diabetes mellitus. Both these conditions are particularly dangerous and burdened by significant mortality, particularly in patient in which the state of diabetes was previously unknown.

For this reason, in patients with diabetes mellitus with severe UC, in addition to a close monitoring of bowel disease which requires clinical evaluation, dosage of C-reactive protein, blood counts and abdominal X-ray studies, a careful evaluation of the diabetes is also required, through the regular monitoring of blood sugar and various blood parameters (electrolytes with evaluation of the anion gap and osmolality, phosphorus, magnesium, creatinine, urinalysis to evaluate ketones, blood-gas analysis to evaluate arterial or venous pH), in particular in patients with basal glycaemia exceeding 180 mg/dL (Table 3).

Therapy of this condition is essentially based on rehydration with saline solution, correction of blood glucose by administering intravenous or subcutaneous insulin and treatment of hypokalemia by re-integration of potassium, bicarbonates, magnesium and phosphate

(in diabetic ketoacidosis), in close collaboration with endocrinologist. Hypokalemia and hypomagnesemia are important risk factors for toxic megacolon^[53], require a prompt correction and suggest careful radiographic and biochemical monitoring, particularly when a septic condition coexists (Table 3).

Therefore, in diabetic patients with acute severe UC, close monitoring of the patient's clinical and biochemical condition is essential, in order to timely identify the opportunity of a second line medical treatment or the possible need for surgical treatment (Table 3). However, the outcome of severe UC in diabetics is still unknown.

In diabetic patients with unstable blood glucose control, steroid treatment can be replaced by intravenous cyclosporine (4 mg/d), infliximab (5 mg/kg or 10 mg/kg at 0, 2, 6 wk and then every two months) or adalimumab 160 mg/80 mg/40 mg eow^[49]. Efficacy and safety of cyclosporine and infliximab are comparable and, in clinical practice, the treatment choice should be guided by physician and centre experience^[54]. A study by Moskovitz *et al.*^[55] showed that cyclosporine is less effective in patients treated with azathioprine and should be avoided in patients with low cholesterol or magnesium in view of the increased incidence of neurological side effects in this patient group. Furthermore, there is no scientific evidence on the effectiveness of cyclosporine in preventing colectomy^[56], while other studies have shown that Infliximab can reduce the rate of colectomy compared with placebo^[57]. So the choice should be placed on individual circumstances and the availability of drugs.

The use of tacrolimus, as an alternative to steroid therapy in diabetic patients, is generally not recommended. The tacrolimus, infact, besides being unable to induce significant mucosal healing compared with placebo^[58], can induce long-term hyperglycemia or even diabetes, as well as hypomagnesemia and it may promote the onset of opportunistic infections^[49].

In contrast, leucocyapheresis, whose principle is based on the extracorporeal removal of leukocytes through an adsorptive system of cellulose acetate beads (Adacolumn, Otsuka Pharmaceuticals) or a polyester fibre filter (Cell-sorba, Asahi Medical Company) represents a potential therapeutic remedy with a good safety profile, serious side effects being very rare. Leucocyapheresis can be associated with any medical treatment, but the real effectiveness of this device in acute severe UC remains to be determined^[59].

Leucocyapheresis has a wide-spread acceptance in Japan, but its cost may limit its use and its future role in Europe will depend on the outcome of controlled trials^[49]. On this regard, clinical efficacy outcomes are variable, being encouraging in some studies and disappointing in others, and the answer might ultimately lie in the patients' disease status at entry. Patients with the first UC episode and short duration of disease or a fair level of intact mucosal tissue, seem to respond and can be spared from multiple drug therapy. Patients with extensive loss of mucosal tissue and those with a long history of exposure to multiple drugs, like corticosteroids, are

unlikely to respond^[60].

Other therapies such as vedolizumab, visilizumab, abatacept, tofacitinib, which are characterised by different mechanisms of action are currently under investigation and have no role in clinical practice to date^[61].

Mild-moderate UC

Treatment of mild-moderate UC depends on the site of disease.

The first line therapy of mild-moderate distal colitis is topical mesalazine and/or oral mesalazine, in once daily administration, which is as effective as divided doses. Combining topical mesalazine and topical steroids, such as beclomethasone dipropionate, also helps. Patients who fail oral/topical mesalazine and topical steroids should be treated with the addition of oral prednisolone.

In proctitis, it has been shown that topical mesalamine has a higher efficacy on symptoms and endoscopic resolution of the damage compared to topical steroids, generally using suppositories that are more appropriate than enemas. However, topical steroids may be used in substitution of mesalazine, if it is poorly tolerated, or in association with the latter. Indeed, the combination of beclomethasone dipropionate (3 mg) and mesalazine (2 mg) is able to induce an improvement in clinical, endoscopic and histologic proctitis, significantly superior to treatment with mesalazine alone^[62].

In diabetic patients with mild-moderate distal UC, systemic steroid therapy should be avoided by resorting to rectal or oral administration of mesalazine and/or beclomethasone dipropionate or to new therapies such as budesonide Multi-Matrix System (MMX), fluticasone or prednisolone metasulphobenzoate.

In treatment of extensive or distal colitis it is generally recommended, as first choice, a combination of oral and topical mesalazine^[49,63,64]. In fact, the use of mesalazine alone, although at high doses or in controlled release formulation (MMX), gives a percentage of remission of at least 41% and a partial response, not exceeding 72%. Furthermore, in many patients the occurrence of relapse of intestinal disease is not uncommon, even during an appropriate maintenance therapy with mesalazine. In these circumstances the use of systemic steroid therapy is generally recommended, even in non severe forms of colitis. In the diabetic patient, this treatment requires the same level of attention already mentioned for patients with severe colitis.

Controlled colonic release formulations of steroids and steroids equipped with low bioavailability, such as beclomethasone dipropionate, budesonide MMX, fluticasone or prednisolone metasulphobenzoate represent a potential therapeutic resource with the advantage of the absence or the minimisation of the side effects of other steroids, with respect to the suppression of the hypothalamic-pituitary-adrenal axis^[65].

Although there are no specific studies on the undesirable effects of this kind of steroids in diabetic patients with UC, it should be noted that, in both normal sub-

jects and elderly diabetic patients under dietary control, treatment with topical inhaled beclomethasone in high doses for 2 wk, did not produce significant changes in blood glucose and lipid metabolism^[66].

In addition, in both adults and pediatric patients, for whom steroid treatment of UC is the remedy of choice, the oral administration of beclomethasone dipropionate is well tolerated and induces a rapid clinical and endoscopic remission in mild to moderate UC, comparable to mesalazine^[67-69].

Budesonide MMX, 9 mg daily, is effective for the induction of remission of mild-moderate active UC. It is a novel oral formulation of budesonide that uses MMX technology to extend release to the colon, without notable increases in glucocorticosteroid-related side effects, probably due to low bioavailability and to targeted delivery of drug^[70].

In patients with UC fluticasone has negative results, but prednisolone metasulphobenzoate, by oral or topical administration, appears to be effective in active distal UC and in mild-to-moderate UC with a lower incidence of systemic adverse effects in comparison with other glucocorticosteroids^[71].

If systemic steroid treatment is necessary, the occurrence of hyperglycemia (particularly in patients with known diabetes) can be corrected or controlled by rapid-acting insulin, usually administered in the preprandial period (generally at a dose of 0.1 U/kg) or by using biguanides, such as metformin, or thiazolidinediones (also known as glitazones), drugs just approved for the treatment of diabetes mellitus type 2.

Metformin, and in particular the increase in dosage of this drug, is often burdened with gastrointestinal side effects such as nausea, vomiting, anorexia, diarrhea, abdominal pain. These symptoms are usually dose-related and occur especially at the beginning of therapy. In 3%-5% of cases, diarrhea may be persistent and cause discontinuation of the drug.

In contrast, the thiazolidinediones are oral antidiabetics with anti-inflammatory properties, potentially useful in patient suffering from UC. In particular, these drugs work by binding to the gamma sub-unit of PPAR (peroxisome receptors that trigger proliferation), receptors located inside the cell nucleus, abundantly expressed in adipose tissue and in the colonic epithelium. Experimental evidence has shown that these molecules have anti-inflammatory activity, in particular in colon and that treatment with these drugs is able to attenuate the production of inflammatory cytokines and to reduce inflammation in animal models of colitis. One uncontrolled study showed that rosiglitazone (a drug used in the United States for treatment of diabetes mellitus type 2) is able to improve moderately active colitis, refractory to treatment with mesalazine. The same group later confirmed the efficacy of the drug, compared to placebo, in a double-blind controlled study^[72,73]. Unfortunately, rosiglitazone was recently withdrawn from the market in some countries for the high risk of ischemic heart

disease and myocardial infarction, but other thiazolidinediones are on the market (such as pioglitazone) or should soon enter in the pharmaceutical reference book and could be used in diabetic patients with UC, if their efficacy in UC will be confirmed.

CONCLUSION

Diabetes mellitus is a common disorder, significantly associated with UC. Co-morbidity among these autoimmune disorders and familial associations with several autoimmune and related diseases, suggest a genetic sharing but, although some shared loci at risk have been identified, the clinical implications of this findings are still unclear.

Likewise, diabetes mellitus and UC share neurological, hepatobiliary, osteoarticular, vascular and post-operative complications. Their onset may be increased by the long-standing concomitance of both diseases. Although specific studies on this aspect have not yet been carried out, this deserves attention in clinical practice. In particular, the role of hyperglycaemia and the poor control of blood glucose level in diabetics deserve particular attention for the risk of morbidity of patients undergoing ileoanal pouch after proctocolectomy.

One of the most common and challenging problems in diabetic patients with UC is the medical treatment. Corticosteroids, the treatment of choice of active UC, may be associated with the onset of glucose intolerance and diabetes, and with the difficult control of blood glucose levels and complications in diabetic patients. Advanced age, high body mass index, family history of diabetes or previous gestational diabetes should always suggest the need of monitoring blood glucose level during steroid therapy. Likewise, rehydration, correction of blood glucose and hypokalemia in close collaboration with endocrinologists, as well as the close monitoring of the patient's clinical and biochemical conditions are essential in diabetic patients with acute UC. Moreover, the potential negative effects of metformin and the beneficial effects of thiazolidinediones on symptoms of UC in remission, should be considered.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Hepatitis B and C virus reactivation in immunosuppressed patients with inflammatory bowel disease

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Abstract

In recent years, a number of case reports and clinical studies have highlighted the risk of hepatitis B and C virus reactivation in patients with inflammatory bowel disease who are treated with immunosuppressive drugs. The cases of viral hepatitis reactivation that have been reported are characterized by a wide range of clinical manifestations, from viremia without clinically relevant manifestations to fulminant life-threatening hepatitis. The development and dissemination of biological immunosuppressive drugs have led to a significant increase in the number of reports of interest to physicians in a variety of clinical settings. On this topic, there have been a number of published guidelines and reviews that have collected the available evidence, providing recommendations on prophylactic and therapeutic strategies and methods for monitoring patients at risk. However, it should be noted that, to date, very few clinical studies have been published, and most of the recommen-

dations have been borrowed from other clinical settings. The published studies are mostly retrospective and are based on very heterogeneous populations, using different therapeutic and prophylactic regimens and obtaining conflicting results. Thus, it seems clear that it is desirable to concentrate our efforts on prospective studies, not conducting further reviews of the literature in the continued absence of new evidence.

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Key words: Inflammatory bowel disease; Biological agents; Hepatitis B virus reactivation; Hepatitis C virus reactivation; Prophylaxis

Core tip: Our review focused on the redundancy of papers on hepatitis B virus and hepatitis C virus reactivation in patients undergoing immunosuppressive therapy. However, we emphasize that, to date, very few clinical studies have been published, and most of them were retrospective with conflicting results. Thus, it is essential to conduct prospective studies before performing additional reviews of the literature.

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INTRODUCTION

In recent years, a significant number of warnings have been given about the risk of hepatitis B and C reactiva-

tion in patients receiving immunosuppressive therapy in different clinical settings (oncology, hematology, solid organ transplant, rheumatology, gastroenterology, and dermatology).

In particular, a number of case reports and clinical studies have highlighted the risk of hepatitis virus B and C reactivation in patients with inflammatory bowel disease (IBD) treated with immunosuppressive drugs^[1-7].

The cases of viral hepatitis reactivation that have been reported are characterized by a wide range of clinical manifestations, from viremia without clinical relevant manifestations to fulminant life threatening hepatitis.

The risk and severity of clinical reactivation seems to be related to the type of immunosuppressive drug administered (steroids, traditional immunosuppressants, or biologics). The various reports and clinical studies on this topic describe and analyze patient populations subjected to different treatment regimens that are not comparable. Moreover, these studies have heterogeneous results and conclusions^[8-13].

The magnitude of the “reactivation” problem seems much more relevant to the hepatitis B virus (HBV) than to the hepatitis C virus (HCV). To date, many reviews comparing few clinical trials have been published on this issue. Among the clinical trials, there was only one prospective study. All of the studies were conducted in different populations with different methods and drawing varying conclusions. Furthermore, the definitions of HBV and HCV reactivations used vary significantly among these studies. Some studies have considered only the increase of viral load, while others have considered the increased viral load in association with elevated levels of aminotransferases.

While some recommendations may appear “evidence-based”, such as prophylaxis for hepatitis B surface antigen positive (HBsAg+) patients receiving immunosuppressive therapy, there are large gray areas, indicating the risk of reactivation in patients with hepatitis B core antibody (HBcAb) isolated or and HCV positivity.

As mentioned above, there is currently not enough strong evidence regarding the prevalence and clinical impact of hepatitis B and C virus reactivation in patients with IBD who are receiving immunosuppressive therapy. This finding calls for the development of prospective clinical trials that can respond to the growing demand of evidence on this issue^[14,15].

IMMUNOSUPPRESSIVE THERAPY IN IBD

The ideal therapeutic approach to IBD should be aimed at inducing and maintaining long-term clinical remission with the minimal use of steroids and surgical interventions; this end-point is particularly important for patients affected by Crohn's disease (CD).

However, about half of IBD cases show a steroid-dependent or steroid-refractory clinical course^[16,17]. Currently, a traditional immunosuppressive therapeutic regimen is indicated for these patients and is mainly represented

by the use of azathioprine and 6-mercaptopurine, even if this regimen produces a low percentage of clinical remission (50%) in the treated population^[18,19]. Other potentially effective immune-suppressors are methotrexate, cyclosporine and tacrolimus. These drugs are rarely used in this setting due to the lack of significant evidence on their efficacy and are used in clinical practice for steroid-refractory ulcerative colitis (UC), as well as anti-tumor necrosis factor alpha (anti-TNF α) agents^[20-30].

IBD patients who are refractory or intolerant to azathioprine are the main candidates for biological anti-TNF α drugs (*i.e.*, adalimumab and infliximab).

Infliximab has proven to be an effective drug in inducing and maintaining clinical remission in refractory luminal and fistulizing CD, with a remission rate of approximately 50%^[29,31-36]. The efficacy of infliximab in treating UC is less impressive than in CD. However, the ACT-1 study^[37] reported an efficacy of 39% and 20% for infliximab in inducing the remission of steroid-dependent UC at weeks 8 and 54, respectively. Furthermore, this drug can avoid the 3-mo need for colectomy in approximately 65% of patients who experience a severe attack of UC^[38,39].

At present, adalimumab, a fully human recombinant anti-TNF α antibody, is approved for the treatment of CD and UC. This drug, administered by subcutaneous injection, has been efficient in inducing and maintaining remission in CD, with a 40% remission rate at week 56^[40,41]. Therefore, similar to infliximab, adalimumab has proven to be valuable in reducing the need for steroids and surgery^[42].

In view of their high efficacy, it has also been suggested that biologics could be used in the early phases of IBD in accordance with a top-down therapeutic strategy^[43], particularly for patients with a poor prognosis and factors implicating a potentially aggressive disease (*i.e.*, young age, smoking, perianal/rectal disease in CD, extensive small bowel involvement, extra-intestinal manifestations, and steroid use at diagnosis)^[42,44-47].

PREVALENCE OF HBV AND HCV INFECTION IN PATIENTS WITH IBD

The prevalence of HBV infection varies greatly throughout the world, from the low rate of < 1% to the moderate rate of 1%-2% in Western countries, towards a much higher rate of > 8% in Asia and in most parts of Africa. The dramatic progress in controlling the spread of HBV in Western countries is prevalently due to the implementation of the vaccination and the adoption of satisfactory measures in preventing HBV transmission^[48-53].

Patients with IBD are considered at risk of HBV and HCV infection because of the frequent need for surgical, endoscopic and transfusion procedures, suggesting the existence of nosocomial transmission. On the other hand, the magnitude of this risk is unknown because there are conflicting reviews and little information on this topic.

Five studies from Italy^[8,54], France^[49], Spain^[55] and China^[51] have evaluated the prevalence of HBV and HCV infections in consecutive series of patients with IBD. Between 1997 and 1999, 332 patients affected by CD were enrolled in an Italian case-control study published in early 2000. The prevalence of HBsAg, HBcAb and HCV in this cohort was 2.1%, 10.9% and 7.4%, respectively. Ten years later, the prevalence of HBsAg and HCV was approximately 1% (Table 1) in 315, 2076 and 301 consecutive subjects in three series of French, Spanish and Italian IBD patients. HBcAb positivity was present in 8/315 (2.54%) of patients in the French study (3 HBsAg+ and HBcAb+; 2 isolated HBcAb+; and 3 HBcAb+ and anti-HBs+); in 154/2056 (7.49%) of patients in the Spanish study; and in 22/301 (7.31%) of patients in the Italian study.

Therefore, the prevalence of HBV and HCV infection in IBD patients seems to be lower than expected, similar to the general population. These results indicate that IBD patients in Western European countries should no longer be considered as a risk group for HBV or HCV infection.

However, a recent study conducted in China on 714 IBD patients showed that the prevalence of HBV infection in IBD patients was higher than reported in the control group. Indeed, the cumulative prevalence of HBsAg and HBcAb was 40.62% *vs* 27.58% in non-IBD patients. The prevalence of HCV infection was similar to that found in non-IBD patients (0.42% *vs* 0.36%).

HBV INFECTION

Mechanism of reactivation

From an immunological point of view, a hepatitis flare rarely starts during the phase of maximal immunosuppression. The majority of reported HBV reactivations matured at the time of the withdrawing or tapering of immunosuppressive therapy, when the immune system has been able to react to the viral replication and to destroy infected hepatocytes^[56].

In this perspective, any deficiency of the immune response to infections caused by immunosuppressive/chemotherapeutic drugs would play a crucial role in disease progression.

In addition, several *in vitro* and *in vivo* animal studies have demonstrated that TNF- α plays a key role in clearing HBV from infected hepatocytes. Hepatitis B viral HBx proteins sensitize the cells to apoptotic killing by TNF, which is secreted by cytotoxic T lymphocytes, together with Interferon- γ (IFN- γ). Both TNF and IFN- γ clear HBV replicative intermediates from the cytoplasm and covalently close circular DNA from the nuclei of infected hepatocytes^[57,58].

Based on these results, it is possible that the use of anti-TNF α drugs in patients with chronic HBV infections could cause an increase in viral replication, leading to liver immune-mediated damage when the inhibitory effect of therapy disappears^[59].

According to this pathogenic model, the risk of HBV reactivation is closely related to the level of immunosuppression achieved, which changes significantly based on the immunomodulatory agents used during the therapy. Stronger immunosuppression may lead to increased viral replication; consequently, a more severe clinical reactivation may occur when immunosuppressive therapy is discontinued^[56].

Immunosuppressive therapy and risk of reactivation in HBsAg positive patients

The reactivation of HBV is an important concern for patients taking immunosuppressants because of the many ways it can affect the body, from a subtle shift in serum aminotransferase levels to fulminant hepatic failure and/or death.

The reactivation of HBV infection (with liver dysfunction, including fulminant hepatitis) is a well-described complication of immunosuppression in the setting of organ transplantation or cancer chemotherapy, occurring in approximately 50% of patients for whom concomitant anti-viral therapy is not used. Mortality from fulminant liver failure after the reactivation of HBV in patients receiving chemotherapy is reported in 4%-60% of cases. The traditional immunosuppressive drugs used in the management of IBD patients include the following: corticosteroids; thiopurines: azathioprine and 6-mercaptopurine; calcineurin inhibitors; cyclosporin and tacrolimus; and methotrexate. Moreover, in the past decade, these biological agents, especially TNF α inhibitors, have been used worldwide and are particularly beneficial in the management of complex and fistulizing diseases^[19-23,31-36].

From a pathogenic perspective, the risk of HBV reactivation is closely related to the levels of immunosuppression. The use of conventional immunosuppressive drugs determines low levels of immunosuppression and does not seem to be associated with the risk of HBV reactivation; this claim is supported by the results of only one study^[9]. In a series of 332 CD patients, only 4 HBsAg positive patients were treated with conventional immunosuppressive drugs (2 with azathioprine and 2 with corticosteroids) and followed-up for at least 1 year. No influence on the clinical course of HBV infection and no episodes of viral or biochemical reactivation were observed in this series of patients over the follow-up of 12 mo.

Notably, only a few cases (4 cases) of HBV reactivation during conventional immunosuppressive therapy (prednisone and azathioprine) resulting in fulminant hepatic failure has been reported in the literature^[11,60].

In recent years, a growing number of cases of HBV reactivation among patients with IBD treated with TNF- α -inhibitors have been described. The available data are limited to a small number of single case reports and a very small series of consecutive patients^[10,12-13,60-65].

Eight case reports describe the use of anti-TNF α drugs in patients with IBD infected with HBV (HBsAg+

Table 1 Virologic and clinical outcomes of hepatitis B virus infection in patients with inflammatory bowel disease undergoing immunosuppressive therapy

Ref.	Disease	Age/sex	HBsAg status	HBV-DNA before therapy	Anti-TNF α	Contemporary drugs	LAM prophylaxis	HBV-DNA reactivation	Biochemical reactivation
Esteve <i>et al</i> ^[66]	CD	34 M	+ IC	NA	IFX	AZT	No	Yes 10.400 pg/mL	ALT 2089 AST 1561
	CD	38 M	+ IC	NA	IFX	AZT	No	Yes 9000 pg/mL	ALT 2225 AST 2146
	CD	26 M	+ CH	Positive	IFX	AZT	Yes	No	No
del Valle <i>et al</i> ^[61]	CD	40 M	+ CH	Positive	IFX	AZT	No	No worsening	No
				3.9×10^5 copies/mm ³					
Ueno <i>et al</i> ^[64]	CD	28 F	+ IC	NA	IFX	AZT	No	Yes 4.5 LEG/mL	ALT 43 AST 64
Millonig <i>et al</i> ^[12]	CD	50 M	+ IC	Positive	IFX	AZT	No	Yes 38000000 UI/mL	ALT 983/50 AST 413/50
Colbert <i>et al</i> ^[10]	CD	54 M	+ IC	NA	IFX	AZT	No	Yes 1.604 pg/mL	ALT 124 AST 143
Madonia <i>et al</i> ^[13]	CD	41 F	- OC	NA	IFX	Steroids	No	Yes	ALT \times 10 UNL AST \times 6 UNL
Ojito <i>et al</i> ^[63]	CD	43 F	+ IC	NA	IFX	AZT	No	Yes 5.4 LGE/mL	ALT 239 AST 145
Zeitz <i>et al</i> ^[11]	UC	43 M	NA	NA		Steroids + AZT	No	Yes 110000000 UI/mL	ALT 3396 AST 2193

CD: Crohn's disease; UC: Ulcerative Colitis; IC: Inactive carrier; OC: Occult carrier; NA: Not available; CH: Chronic hepatitis; IFX: Infliximab; AZT: Azathioprine; LGE: Logarithm genome equivalent; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; TNF α : Tumor necrosis factor alpha; LAM: Lipoarabinomannan.

inactive carriers). All were affected by CD, and 7 out of 8 did not receive immunoprophylaxis. Only one patient received lamivudine as a prophylactic treatment. Six out of 7 showed HBV reactivation with a wide range of outcomes, ranging from a modest increase in the viral load and ALT levels to fatal hepatitis. Interestingly, the only patient who received prophylactic treatment did not experience viral, biochemical or clinical reactivation during the 6 mo follow-up^[66]. The duration of anti-TNF α therapy before HBV relapse varied from a single infusion to many months of treatment, and all of the episodes of reactivation were observed in patients receiving infliximab and other immunosuppressive drugs at the same time (Table 1). No reports are available in patients using adalimumab.

Recently, Loras *et al*^[55] recorded all clinical information from IBD patients with HBV and HCV infections in 19 Spanish hospitals. HBV reactivation was observed in 9 out of 25 (36%) HBsAg+ patients. All but two cases were treated with simultaneous immunosuppressors [steroid + azathioprine (4 cases) and infliximab + azathioprine (3 cases)]. In contrast, the patients without reactivation were only treated with one immunosuppressant and/or received prophylactic antiviral treatment. In 6 out of the 9 cases (66%), HBV reactivation caused severe hepatic failure.

On the contrary, Morisco *et al*^[65] conducted a retrospective multicenter study, including 5096 patients with IBD. HBV reactivation was observed in only 1 out of 6 (16%) HBsAg+ patients undergoing immunosuppressive therapy. A combined immunosuppressive therapy had been administered in all cases of HBV reactivation (Table 2).

Immunosuppressive therapy and risk of reactivation in HBsAg negative/HBcAb positive carriers

HBsAg negative and HBcAb positive IBD patients seem to have a low risk of HBsAg seroreversion and hepatitis flares. Indeed, in opposition to the results found in other clinical settings, only one case of HBV reactivation has been described in an IBD patient^[13]. A female CD patient was treated with 25 mg/d of prednisone and infliximab for the relapse of disease after an unsatisfactory remission with conventional therapy (steroids, ciprofloxacin, metronidazole, and methotrexate). After a month of treatment with infliximab and steroids, the patient's aminotransferases increased (10 times over the upper normal limit), together with the emergence of HBsAg, hepatitis Be antibody, HBcAb, IgM HBcAb, and HBV-DNA positivity in the serum.

HCV

Immunosuppressive therapy and risk of reactivation

To date, there is no conclusive information on the safety of immunosuppressive or immunomodulator drugs in HCV among IBD patients. These patients appear to be at low risk, although long-term safety studies are needed.

There are several interesting concerns regarding immunosuppressive treatment for IBD in patients with HCV infection. For example, prednisone, which is frequently used to treat acute exacerbations of intestinal diseases, may negatively affect HCV infection by increasing the viral load^[67-69].

On the other hand, anti-TNF α drugs seem to reduce inflammation through TNF α inhibition, playing a role in the pathogenesis of HCV^[70].

Table 2 Reactivation in patients with inflammatory bowel disease undergoing immunosuppressive therapy

Ref.	HBsAg+	HBcAb+	HCV+
Loras <i>et al</i> ^[60]	9/25	0/65	8/51
Morisco <i>et al</i> ^[65]	1/6	1/4	1/10
Papa <i>et al</i> ^[54]	0/1	0/22	0/4

HBsAg: Hepatitis B surface antigen; HBcAb: Hepatitis B core antibody; HCV: Hepatitis C virus.

Few studies have been performed to evaluate the safety of TNF α antagonist medications in IBD patients with HCV, and most of these are case reports or small case series^[54,55,65].

The larger series available on this topic show a low risk of hepatitis flares in these patients with a mild-moderate clinical course^[54,60,65]. Indeed, HCV reactivation was observed in 8/51 (15.7%) and in 1/10 (10%) HCV-RNA positive patients, respectively, by Loras *et al*^[60] and Morisco *et al*^[65]. All cases of reactivation had a very mild course, except for one patient, who died.

The deceased patient described by Loras *et al*^[60] and experienced severe liver failure. He died while receiving steroids. Importantly, he also had an occult HBV infection and was human immunodeficiency virus-positive.

SCREENING AND VACCINATION

Screening measures must be instituted in IBD patients. Recommendations are based on the potentially fatal consequences of HBV and HCV reactivation and the availability of safe and effective drugs to prevent these situations^[11].

HBV

The most recent guidelines of the European Crohn's and Colitis Organization (ECCO) on the management of opportunistic infections in IBD^[17] recommend that all IBD patients be tested for HBsAg, HBcAb and HBsAb to assess their infection or vaccination status; vaccination is recommended in all seronegative patients.

At present, only four studies have evaluated the HBV vaccination status of patients with IBD. Evidence of effective vaccination (positive anti-HBs and negative HBcAb) was only detected in 12%, 48.9%, 24% and 21.7% of the four cohorts of patients enrolled in Spain, France, Italy and China, respectively^[49,51,54,55]. No information about the adherence to HBV vaccination is available in the literature for other series of European patients with IBD.

HBV vaccination coverage significantly differs among European countries because the vaccination programs were started in different years and have been proposed for different target populations (newborns, adolescent and pre-adolescent subjects, only for high-risk groups, *etc.*)^[71].

As consequence, the determination of the infectious or vaccination status at the time of a diagnosis of IBD

seems to be appropriate. An administration of the vaccine in negative subjects (HBsAg, HBcAb and HBsAb negative) as soon as possible should progressively reduce the number of cases with problematic management. Improved adherence to the program of universal vaccination will reduce the need for screening in the future.

HCV

No recommendations have been proposed for HCV screening prior to starting immunomodulators by the ECCO guidelines^[72]. However, we do believe that HCV screening (including HCV antibodies and HCV-RNA if anti-HCV+) should be routinely performed upon the completion of liver function tests before starting immunosuppressive therapy. If positive, these tests should be performed again every 3 mo to carefully monitor the patient's status during immunosuppressive treatment.

With regard to the use of anti-TNF α agents, it is important to emphasize that their use should be evaluated on the basis of the clinical underlying condition of the patient. In particular, while the use of anti-TNF α in non-cirrhotic patients appears safe, it is contraindicated in patients with decompensated cirrhosis and should be used with caution in patients with compensated cirrhosis on a case-by-case basis, according to the benefit/risk ratio.

PROPHYLAXIS AND THERAPY

Several studies have reported a preventive effect of antiviral agents on hepatitis B reactivation during immunosuppression therapy, and specific recommendations were elaborated by American Association for the Study of Liver Diseases, European Association for the Study of the Liver and Association for the Study of the Liver^[59,73,74]. Nonetheless, significant questions regarding the optimal antiviral prophylaxis strategy have yet to be addressed.

The effect of prophylactic antiviral therapy on the course of HBV infection in IBD patients undergoing immunosuppressive therapy has not been studied prospectively, and, until now, only a few studies have reported a good efficacy in a short period of follow-up^[65,66]. As a result, the management strategy for these patients remains uncertain.

Lamivudine prophylaxis was suggested on the basis of its well demonstrated efficacy; however, other nucleotides/nucleosides should be preferred, especially if immunosuppressive therapy is scheduled for more than 12 mo, due to their lower propensity to provoke drug resistance. Conversely, HBsAg- HBcAb+ patients (with or without HBsAb+), given their low risk for reactivation, do not require routine antiviral prophylaxis, but only periodic monitoring (about every 3 mo) for the elevation of aspartate aminotransferase/alanine aminotransferase and HBsAg, to prove the re-emergence of HBV-DNA^[72].

Prophylactic therapy seems to be appropriate when biological therapy is scheduled, especially when combined with other immunosuppressive drugs. The role of antiviral drugs other than lamivudine, including entecavir, ad-

efovir and tenofovir, is still unknown, and further studies are needed^[75].

To date, there is no prophylaxis available for HCV reactivation. Interferon, which is currently the milestone of antiviral therapy for HCV, is contraindicated in IBD forms that require immunosuppressive therapy. The availability of interferon-free regimens could dramatically change this scenario.

CONCLUSION

The issue in question has increasingly gained interest over the past few years, with the emergence of a number of reports (case reports or case series) on the risk of reactivation of hepatitis in immunosuppressed patients with IBD.

The first cases reported in literature date back to the 1990s and studied onco-hematological patients who demonstrated a fulminant HBV reactivation during or after chemotherapy treatments.

In subsequent years, the development and dissemination of biological immunosuppressive drugs led to a significant increase in the number of reports aimed at reaching other clinical settings (dermatology, rheumatology, gastroenterology, *etc.*).

In particular, the use of anti-TNF α is more frequent in the treatment of steroid-dependent and steroid-refractory UC and CD, as well as in the treatment of perianal fistulizing CD or extraintestinal manifestations associated with IBD (ankylosing spondylitis, pyoderma gangrenosum and uveitis).

There have been a number of published guidelines and reviews on this topic that have collected the available evidence and provided recommendations on prophylactic and therapeutic strategies and methods for monitoring patients at risk.

However, it should be noted that, to date, very few clinical studies have been published, and most of their recommendations have been borrowed from other clinical settings. The published studies are mostly retrospective and based on heterogeneous populations, using different therapeutic and prophylactic regimens to obtain conflicting results.

In particular, the two studies with larger series are those of Loras *et al.*^[60] and Morisco *et al.*^[65]. The first group analyzed a population of 25 HBsAg+ and 51 HCV-RNA+ patients. Among HBsAg+ patients, 36% experienced a reactivation of the HBV, and 6 of them developed acute liver failure; however, no reactivation was observed in patients with HBsAg-HBcAb positivity. On the other hand, Morisco *et al.* showed a different result in their study. Only 1/6 HBsAg+ (16%) and 1/4 HBcAb+ isolated (25%) had viral reactivations with mild clinical courses.

The two groups of authors agreed on the low risk of HCV reactivation. The study of Morisco *et al.*^[65] on the screening procedures for HBV and HCV in patients with IBD resized the frequency and severity of the clinical im-

pact of viral reactivation in patients with IBD receiving immunosuppressive therapy.

Today, there are still some gaps in the knowledge regarding this clinical area. Current evidence supports the assumption that the HBsAg+ patients should receive prophylactic antiviral therapy prior to initiating immunosuppressive therapy. Nevertheless, the best strategy to adopt in HCV+ or in isolated HBcAb+ patients remains unclear. In these cases, the risk of viral reactivation seems to be low, compared to the former scenario.

Thus, the current evidence suggest that it is essential to place an increased emphasis on the completion of prospective studies and to discourage further reviews of the literature until new evidence is available.

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Clinical management of inflammatory bowel disease in the organ recipient

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Abstract

There was estimated a higher incidence of *de novo* inflammatory bowel disease (IBD) after solid organ transplantation than in the general population. The onset of IBD in the organ transplant recipient population is an important clinical situation which is associated to higher morbidity and difficulty in the medical therapeutic management because of possible interaction between anti-reject therapy and IBD therapy. IBD course after liver transplantation (LT) is variable, but about one third of patients may worsen, needing an increase in medical therapy or a colectomy. Active IBD at the time of LT, discontinuation of 5-aminosalicylic acid or azathioprine at the time of LT and use of tacrolimus-based immunosuppression may be associated with an unfavorable outcome of IBD after LT. Anti-tumor necrosis factor alpha (TNF α) therapy for refractory IBD may be an effective and safe therapeutic option after LT. The little experience of the use of biological therapy in transplanted patients, with concomitant anti-rejection therapy, suggests there be a higher more careful surveillance regarding the risk of infectious diseases, autoimmune diseases, and neoplasms. An increased risk of colorectal cancer (CRC) is present also after LT in IBD patients with primary sclerosing

cholangitis (PSC). An annual program of endoscopic surveillance with serial biopsies for CRC is recommended. A prophylactic colectomy in selected IBD/PSC patients with CRC risk factors could be a good management strategy in the CRC prevention, but it is used infrequently in the majority of LT centers. About 30% of patients develop multiple IBD recurrence and 20% of patients require a colectomy after renal transplantation. Like in the liver transplantation, anti-TNF α therapy could be an effective treatment in IBD patients with conventional refractory therapy after renal or heart transplantation. A large number of patients are needed to confirm the preliminary observations. Regarding the higher clinical complexity of this subgroup of IBD patients, a close multidisciplinary approach between an IBD dedicated gastroenterologist and surgeon and an organ transplantation specialist is necessary in order to have the best clinical management of IBD after transplantation.

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Key words: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Primary sclerosing cholangitis; Liver transplantation; Heart transplantation; Renal transplantation; Anti-tumor necrosis factor alpha therapy

Core tip: Inflammatory bowel disease (IBD) in the organ transplant recipient population is an important clinical situation which is associated to higher morbidity and difficulty in the medical therapeutic management because of possible interaction between anti-reject therapy and IBD therapy. IBD course after liver transplantation is variable, but about one third of patients may worsen, needing an increase in medical therapy or a colectomy. About 30% of patients develop multiple IBD recurrence and 20% of patients require colectomy after renal transplantation. Like in the liver transplantation, anti-tumor necrosis factor alpha therapy could be an effective treatment in IBD patients with conventional

refractory therapy after renal or heart transplantation.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a complex chronic inflammatory intestinal disease with a prevalence steadily increasing during the recent years. The management of IBD is leaning towards more complex clinical situations, with possible interactions between the intestinal disease and others organ diseases. The organ recipient population is constantly increasing in the medical specialized centers in the world and it can happen that a patient with a solid organ transplantation has a recurrence of IBD. This is particularly important in the liver transplantation (LT) because there is a close pathophysiological correlation between primary sclerosing cholangitis (PSC) and ulcerative colitis (UC) before the transplant. *De novo* IBD has been reported after solid organ transplantation, with an incidence estimated ten times higher with respect to the expected incidence of IBD in the general population. Therefore, the organ recipient patient may be a new clinical “scenario” for IBD management.

The first objective of this review was to examine the studies present in the English literature (PubMed) about the natural history in organ recipient patients. In particular, we have evaluated: (1) risk of recurrent IBD; (2) risk of *de novo* IBD; (3) need for colectomy; (4) risk of pouchitis; and (5) risk of colorectal cancer. The second objective was to examine the medical therapy for active IBD after organ transplantation.

We have more knowledge of IBD clinical management after LT, but in this review we have evaluated the IBD studies after heart, renal, lung, and intestinal transplantation.

EPIDEMIOLOGY AND CLINICAL FEATURE OF IBD AFTER LIVER TRANSPLANTATION

Recurrent IBD

Singh *et al*^[1] has recently examined the studies on the natural history of IBD after LT for PSC, reporting 609 patients in 14 studies, followed approximately 4.8 years after LT (range, 1.8-7.2 years), about 31% of patients have improvement of IBD activity, 39% of patients do not have a significant change in IBD activity, and 30% of patients develop worsening IBD, requiring intensification of medical therapy and/or surgery. The estimated risk of severe IBD flare up at 5 and 10 years after LT ranged from 39% to 63% and 39% to 98%, with a median time of a flare up around 1 year (range, 0.3-8.6 years)^[2-4]. The

need of colectomy for acute IBD refractory to medical therapy is nearly 9% (range, 0%-21%)^[2-6]. Dvorchik *et al*^[7] observed a significant 3.1-fold increased risk of colectomy due to severe IBD flare up or medically refractory disease after LT compared to IBD patients who did not require LT.

De novo IBD

Wörns *et al*^[8] evaluated 44 patients with IBD after solid organ transplant (SOT) and reported the *de novo* disease: 38 of 44 (86%) cases occurred following LT (23) or combined liver/kidney transplantation (15), 4 (9%) after heart transplantation, and 2 (5%) after kidney transplantation. Riley *et al*^[9] identified 14 patients who developed *de novo* IBD in 6800 cases after liver and kidney transplantation. Twelve (86%) of the patients developed IBD post liver transplant and two (14%) were detected post kidney transplant. The authors estimated a higher incidence of *de novo* IBD after SOT than in the general population (206 *vs* 20 cases per 100000 persons-year). The higher prevalence of IBD post LT in the SOT patients can be related to the strong association between PSC and UC. In these patients, the 10-year risk of *de novo* IBD after LT is estimated to be 14%-30%, with median time to development of approximately 4 years (range, 1.1-7.1 years)^[2-4]. After a median follow-up of 4 years 18/86 (21%) patients who underwent LT for PSC developed IBD. Verdonk *et al*^[3] observed that the *de novo* IBD patients tended to develop disease later in post-transplant period than the patients with pre-existing IBD. Moreover, the patients with *de novo* IBD after LT respond better to medical therapy and none required colectomy.

RISK FACTORS FOR IBD AFTER LIVER TRANSPLANTATION

Clinical activity of IBD

The clinical activity of IBD at the time of LT may be a risk factor for worsening the intestinal disease after LT. In fact, a three-fold higher risk of IBD flare up after LT in patients with active IBD at the time of LT it was observed^[3].

Smoking

Joshi *et al*^[4] evaluated 110 patients underwent LT for PSC. In the multivariate analysis, active smoking at the time of transplant was the only significant risk factor for flare up of IBD post-transplantation (HR = 17; 95%CI: 2-180).

Cytomegalovirus

Cytomegalovirus (CMV) mismatch (seropositive donor, seronegative recipient and CMV infection) have not been associated with the recurrence of IBD after LT^[4,10,11]. CMV mismatch was associated with a 4.5-fold higher risk of *de novo* IBD after LT, but CMV infection is not related to *de novo* IBD post LT^[11].

Therapy of IBD after LT

The therapy with proven efficacy for active IBD may re-

Table 1 Interaction between inflammatory bowel disease therapy and anti-reject therapy in inflammatory bowel disease patients after liver transplantation

Drug	Efficacy		Ref.
	IBD therapy	Anti-reject therapy	
5-ASA	+		[3] [12]
Prednisone	+	+	[12] [21]
Azathioprine	+	+	[2]
Anti-TNF α	+		[15] [16] [13] [14] [18]
Tacrolimus	-	+	[2] [3]
Cyclosporine	+	+	[3]
Mycophenolate	-	+	[22]
Mofetil			[23]

TNF α : Tumor necrosis factor alpha; IBD: Inflammatory bowel disease; 5-ASA: 5-aminosalicylates.

duce the risk of disease exacerbation post-LT (Table 1).

5-aminosalicylates (5-ASA) therapy after LT appears to be protective against the worsening disease activity of IBD, decreasing the flare up and/or colectomy risk about 80%^[3,12].

Azathioprine may reduce the risk of active IBD after transplant. In the study's Haagsma *et al*^[2], a comparison between 55 patients who received azathioprine with the 23 patients did not receive azathioprine, was performed and this showed a significantly higher IBD-free survival for azathioprine group. In particular, at 1, 3 and 5 years after LT, the IBD-free survival rates in patients receiving azathioprine were 96%, 96% and 88%, respectively; while in patients not receiving azathioprine, these value were 87%, 63% and 54%, respectively.

We have little knowledge about the use of anti-tumor necrosis factor alpha (TNF α) therapy for refractory IBD after transplant. To date, there have been only 22 patients treated with anti-TNF for relapsing IBD following LT; this number includes patients with UC, Crohn's disease, indeterminate colitis and pouchitis, treated with infliximab or adalimumab (Table 2)^[13-18]. In our study, we evaluated the efficacy and safety of infliximab therapy in a homogeneous series of four patients with refractory UC following LT, followed for a median time of 18 mo. At week 54, three patients (75%) experienced sustained improvement of IBD. Complete mucosal healing (defined as absence of lesions) was observed in one of three patients (33%). Steroid treatment was successfully withdrawn during infliximab therapy in all patients. Adverse events included only one infection by *Molluscum contagiosum*, which resolved without sequelae. No malignancies were observed in any patient following infliximab therapy. No cases of hepatic rejection were documented. Our results are in line with others studies about the efficacy and

safety of anti-TNF α therapy in patients with refractory IBD following LT, but larger studies are needed to evaluate the safety profile of biological therapy combined with anti rejection treatment.

Anti-reject therapy

Tacrolimus is the principal immunosuppressive agent in SOT, but it has been observed, in retrospective studies, that it may be associated with a four-fold higher risk of post-LT IBD relapse^[2,3]. In patients with tacrolimus for transplant-related immunosuppression, Dvorchik *et al*^[7] found that the risk of relapse of IBD at 1- and 5-year was 13% and 64%, respectively; while, in patients with tacrolimus-free regimens, the risk of IBD was 4% and 10%, respectively. The cause of a possible relationship between tacrolimus and IBD flare up after transplant is not known. IBD results from inappropriate and ongoing activation of the mucosal immune system in the presence of normal luminal flora. Immunosuppression agents may promote infections which may lead to the change bacterial gut flora and decrease the intestinal barrier function^[19]. Moreover, tacrolimus is a strong inhibitor of interleukin-2 production. Deficiency of interleukin-2 can result in T-cell dysregulation, leading to the development of intestinal chronic inflammation^[2,4].

Cyclosporine, like anti-TNF α , is the chosen drug in severe steroid refractory UC. Cyclosporine does not seem to worsen the course of IBD after transplant^[3,10]. In contrast with tacrolimus, the frequency of interleukin-2-expressing T cells was significantly higher with cyclosporine in renal transplant patients^[20]. This may explain in part the different effect of cyclosporine and tacrolimus on IBD course after transplant.

Corticosteroids are effective in acute and chronic prevention of SOT rejection as well as in inducing clinical remission in IBD. Moncrief *et al*^[21] and Navaneethan *et al*^[12] observed that prednisone therapy may favorably modify the course of IBD after LT, but this therapeutic regime is associated to important side effects.

Mycophenolate mofetil (MMF) has proven efficacy in SOT, but its role in IBD is not clear^[22,23]. Moreover, MMF is associated with enterocolitis, which can mimic a IBD flare up.

COLECTOMY POST-LIVER TRANSPLANTATION

Ho *et al*^[24] observed a prevalence of colectomy after LT about 35%. In this Scottish study 7 of 20 patients underwent colectomy with a median time of 3.4 years (range 1.5-6.3 years) following LT^[24]. The indication for colectomy was chronically active severe UC in three patients (43%), colonic dysplasia or colorectal cancer in three patients (43%) and benign stricture of colon in one patient (14%). The study in Cleveland compared 86 patients with UC and LT for PSC with 81 patients with UC and PSC who did not require LT^[25]. The necessity of colectomy was significantly more frequently in the non-LT group

Table 2 Anti-tumor necrosis factor alpha therapy for management of refractory inflammatory bowel disease after liver transplantation

Author	Number LT patients	Clinical outcome	Endoscopic outcome	Adverse events
Sandhu <i>et al</i> ^[13]	6	Response: 67%	-	Systemic lupus erythematosus
Mohabbat <i>et al</i> ^[14]	8	Response: 87.5%	Mucosal healing: 42.9%	Colorectal cancer Oral candidiasis <i>Clostridium difficile</i> colitis Bacterial pneumonia Cryptosporidiosis Post-transplant lympho-proliferative disorder
Lal <i>et al</i> ^[15]	1	Response: 100%	Improvement: 100%	No
El-Nachef <i>et al</i> ^[16]	2	Response: 100%	-	No
Indriolo <i>et al</i> ^[18]	4	Response: 75%	Mucosal healing: 33%	<i>Molluscum contagiosum</i>

than the LT group (76.5% *vs* 34.9%). The percentage of patients which underwent colectomy for steroid dependent/refractory disease was lower in the LT group than patients in the non-LT group (39.9% *vs* 48.4%). Regarding the possible difficulties to surgically pack the J pouch in patients who underwent LT and Roux-en-Y biliary-jejunal reconstruction, Mathis *et al*^[20] did not encounter any problems in the 13 patients operated on.

POUCHITIS POST-LIVER TRANSPLANTATION

The risk of acute pouchitis after LT for PSC ranged from 14% to 66%^[26-28]. The risk of chronic pouchitis ranged from 9.1% to 73.7%^[26-30]. Freeman *et al*^[29] observed that the risk of chronic refractory pouchitis was comparable in patients who underwent LT and those who did not. Therefore, it seems that LT does not increase the risk of pouchitis. With regard to the therapy of pouchitis after LT, Mathis *et al*^[20] evaluated 32 patients who underwent ileal pouch-anal anastomosis (IPAA) for UC and LT for PSC (in 13 patients IPAA followed by OLT). Two-thirds of the patients had pouchitis during follow-up, about half developed a chronic pouchitis that required daily antibiotic therapy. Only one patient needed a defunctioning ileostomy. Anti-TNF α therapy was used in only four IBD patients in three studies for refractory pouchitis after LT for PSC^[13,14,18]. Even though a clinical improvement was observed, the little data available does not allow us to extrapolate any conclusions about the efficacy and safety of biological therapy in patients with chronic refractory pouchitis after LT.

COLORECTAL CANCER POST-LIVER TRANSPLANT

Epidemiology

A large range with rates of risk of colorectal cancer after LT for PSC, from 0 to 31.5 per 1000 person/year, is present in literature in recent years^[4,7,21,25,31-48]. Watt *et al*^[31], reported a cumulative incidence of colorectal cancer (CRC) at 10-years post LT for PSC of 8.2% as compared to 2.6% after LT for non-PSC patients. The CRC risk in PSC patients without IBD after LT is 2.8% at 10-years. There-

fore, the combination of IBD and PSC after LT leads the patient to a higher risk in developing CRC.

Moreover, the CRC risk is increased in patients with long-standing IBD, long-standing LT and extensive colonic involvement^[32,35,45]. In fact, it was observed at 1-, 5- and 10-year, a cumulative incidence of CRC of 3.3%, 6.7%, and 11.8%, respectively, after LT. Regarding the colorectal cancer location, similarly to IBD/PSC patients before LT, the right-side colon is more frequently affected^[39]. There is no clear evidence if the age of the patients at the time of LT is associated with CRC risk^[7,33,35,45]. No significant association between clinical severity IBD and CRC risk after LT was observed^[39,45]. Regarding the use of 5-ASA or ursodeoxycholic acid in the chemoprevention of CRC, it seems that they don't change the cancer risk^[33,39].

Risk factors

The risk of CRC may increase after LT because of errors in mucosa sampling during colonoscopy or perhaps due to the immunosuppression treatment of anti-reject therapy. Loftus *et al*^[42] observed that the CRC rate was 4.4-fold higher after LT, as compared to a historical cohort patient with PSC/IBD who did not undergo LT. However, the role of LT in the risk of CRC in IBD/PSC patients still isn't clear. In fact, while Dvorchik found that LT did not significantly influence the risk of CRC, it was observed that LT may be an independent risk factor for CRC in other studies^[7,35,42,45].

Surveillance

A program of endoscopic surveillance with serial biopsies for CRC is recommended by the European Association for the Study of Liver^[49]. A colonoscopy is suggested every year in IBD/PSC patients after LT. If dysplasia colonic mucosa is found, a colectomy is advised. It has been shown to be a relatively safe procedure in specialized surgical centers^[45,46].

Management

Adjuvant pharmacotherapy with drugs like oxaliplatin has also been shown to be well tolerated in patients post-LT and hepatic graft dysfunction was not documented^[50]. Prophylactic colectomy in selected IBD/PSC patients with CRC risk factors could be a good management strategy in the CRC prevention, but it is used infrequently in

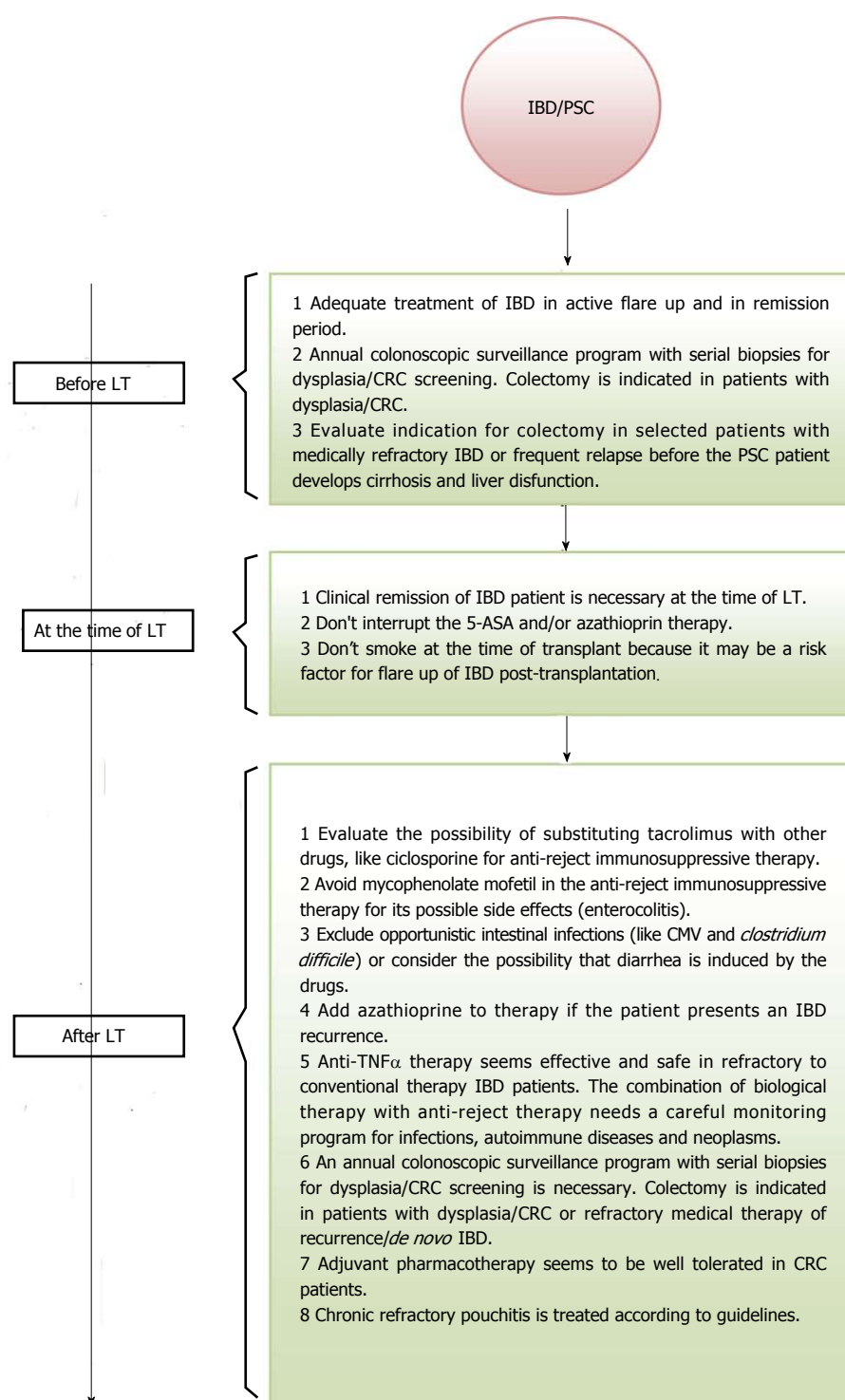


Figure 1 Clinical management of inflammatory bowel disease/primary sclerosing cholangitis patients before and after liver transplantation. TNF α : Tumor necrosis factor alpha; IBD: Inflammatory bowel disease; LT: Liver transplantation; PSC: Primary sclerosing cholangitis; CRC: Colorectal cancer; CMV: Cytomegalovirus; 5-ASA: 5-aminosalicylates.

the majority of LT centers^[45,51].

CLINICAL MANAGEMENT OF ACTIVE IBD AFTER LT

Patients who underwent LT can develop diarrhea and it is very important exclude an intestinal infection (CMV, *Clos-*

tridium difficile), or consider the possibility that diarrhea may be caused by the drugs (Figure 1). If the symptoms of active IBD are confirmed from a colonoscopy and a histological exam, the patient starts the IBD therapy. The plan of IBD therapy in the patient who underwent transplant, is similar to the therapy plan before the transplant. 5-ASA at a dose of 2.4 g per day is indicated in induction and in maintenance in the mild-to-moderate UC patients. Topical

therapy with 5-ASA and/or beclomethasone dipropionate can be useful in distal UC. Budesonide or systemic steroids are indicated in patients with mild Crohn's disease. In moderate-to-severe IBD patients the use of oral or *iv* corticosteroids are necessary. Prednisone at a dose of 50 mg per day or prednisolone at a dose of 40-60 mg per day based on the patient's body weight (60-80 kg) may be used in clinical practice. A gradual tapering of corticosteroids is recommended, for example 5 mg every week in prednisone therapy. Maintenance immunosuppression therapy with azathioprine at a dose of 2.0-2.5 mg/kg body weight per day is effective after the corticosteroid therapy. Anti-TNF α treatment could be effective and safe in refractory to conventional therapy IBD^[13,14,18]. The little experience of the use of biological therapy in transplanted patients, with concomitant anti-rejection therapy, suggests there be a higher and more careful surveillance regarding the risk of infectious diseases, autoimmune diseases, and neoplasms.

It is very important to consider the possibility of surgical treatment in patients with moderate-to-severe IBD after LT for PSC and even more so, because of the higher colorectal cancer risk. Proctocolectomy with IPAA is feasible and safe in dedicated surgical centers^[26,27].

IBD AFTER HEART TRANSPLANTATION

Three cases of *de novo* IBD after heart transplantation have been reported in 3 studies: two cases of Crohn's disease and one case of UC^[52-54]. The onset of IBD has been observed in pediatric age in two of three patients. Rakhit *et al*^[52] reported one case of Crohn's disease in 104 post-orthotopic heart transplant children. The patient developed diarrhea and rectal bleeding immediately after the transplant and IBD was diagnosed after one year. The patient continued to have flares despite immunosuppressive therapy. Harms *et al*^[53] reported a 15-year-old girl developed IBD 10 years after cardiac transplantation and presented a severe growth failure and delayed onset of puberty. The patient was found to have pan-enteric Crohn's disease and has done remarkably well following a nutritional therapy. Jüngling *et al*^[54] reported a 53-year-old patient who developed distal UC 2 years after heart transplantation. In spite of high-dose treatment with prednisolone the patient's clinical situation worsened with a progression of inflammation in the entire colon. Colectomy with ileostomy was necessary to obtain a good state of health. Three IBD cases have been observed after heart transplantation in spite of immunosuppressive therapy. Two of them have been treated with conventional medical therapy with success, whereas one IBD case required surgery with colectomy and ileostomy. No IBD patient was treated with anti-TNF α therapy.

IBD AFTER RENAL TRANSPLANTATION

A total of about of twenty-seven *de novo* IBD patients (15 UC and 11 Crohn's disease) after renal transplantation in

11 studies were reported^[9,16,54-63]. One patient presented erythema nodosum associated to IBD^[60], one patient developed colonic cancer with liver metastasis 2 years later and died^[60], thirteen (50%) patients were treated with conventional medical IBD therapy (mesalazine, corticosteroids, and azathioprine), achieving a clinical remission. Significant clinical improvement of IBD was observed with anti-TNF α therapy in three (11.5%) patients. No severe infections or graft reject were documented after biological therapy^[22,61]. Five (19.2%) patients with Crohn's disease continued to have flare up despite treatment^[9]. Five (19.2%) UC patients were refractory to therapy and required a colectomy^[9,55,56,62].

IBD AFTER LUNG AND INTESTINAL TRANSPLANTATION

The number of patients who underwent lung or intestinal transplantation is significantly lower than the patients who underwent liver, heart, and renal transplantation. This could probably be explained because no IBD cases were reported after lung and intestinal transplantation.

CLINICAL MANAGEMENT OF IBD PATIENTS AFTER HEART AND RENAL TRANSPLANTATION

The concomitant anti-reject immunosuppressive therapy can increase the risk of infectious diseases in transplanted patients. Therefore, in patients who develop diarrhea, it is very important to exclude an intestinal infection (CMV, *Clostridium difficile*). It is also necessary to exclude that diarrhea may be induced by the drugs. The onset of IBD after heart or renal transplantation could lead to a severe clinical situation for the transplanted patient. In fact, in about half of the patients, conventional IBD therapy combined with anti-reject therapy, can be non-effective. Nineteen percent of patients developed multiple recurrence on IBD in the renal transplantation group. Eleven percent of patients required anti-TNF α therapy in order to have clinical remission and in about 20% of patients required a colectomy. The LT model suggests that anti-TNF α therapy combined with anti-reject therapy could be useful in selected IBD patients with refractory to conventional therapy after heart or renal transplantation. Like in the LT, it is very important that there be a higher more careful surveillance regarding the risk of infectious diseases, autoimmune diseases, and neoplasms after biological therapy in heart and renal transplanted patients. The surgical option remains an essential treatment in complicated IBD cases which are refractory to the intensive medical therapy.

CONCLUSION

There is estimated a higher incidence of *de novo* IBD after solid organ transplantation than in the general popula-

tion. The onset of IBD in the organ transplant recipient population is an important clinical situation which is associated with higher morbidity and difficulty in the medical therapeutic management because of the possible interaction between anti-reject therapy and IBD therapy. IBD course after LT is variable, but about one third of patients may worsen, needing increased medical therapy or a colectomy. Active IBD at the time of LT, discontinuation of 5-ASA or azathioprine at the time of LT and use of tacrolimus-based immunosuppression may be associated with an unfavorable outcome of IBD after LT. Anti-TNF therapy for refractory IBD may be an effective and safe therapeutic option after LT. The little experience of the use of biological therapy in transplanted patients, with concomitant anti-rejection therapy, suggests there be a higher more careful surveillance regarding the risk of infectious diseases, autoimmune diseases, and neoplasms. Therefore, it is very important to consider the possibility of a surgical treatment in refractory severe IBD after LT. An increased risk of colorectal cancer is also present after LT in IBD/PSC patient. An annual program of endoscopic surveillance with serial biopsies for CRC is recommended. If dysplasia colonic mucosa is found, a colectomy with IPAA is advised. It has been shown to be a relatively safe procedure in the specialized surgical centers. Adjuvant pharmacotherapy has been shown to be well tolerated in patients post-LT. Hepatic graft dysfunction has not been documented after adjuvant pharmacotherapy. A prophylactic colectomy in selected IBD/PSC patients with CRC risk factors could be a good management strategy in the CRC prevention, but it is used infrequently in the majority of LT centers. About 30% of patients developed multiple recurrences of IBD and 20% of patients required a colectomy after renal transplantation. Like in the LT, anti-TNF α therapy could be an effective treatment in IBD patients with conventional refractory therapy after renal or heart transplantation. A large number of patients are needed to confirm the preliminary observations.

Regarding the higher clinical complexity of this subgroup of IBD patients, a close multidisciplinary approach between an IBD dedicated gastroenterologist and surgeon and an organ transplantation specialist is necessary in order to have the best clinical management of IBD after transplantation.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Pharmacogenetics of azathioprine in inflammatory bowel disease: A role for glutathione-S-transferase?

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Core tip: Polymorphisms of glutathione-S-transferase-M1 may influence azathioprine effects in young patients with inflammatory bowel disease by increasing the drug activation and by modulating oxidative stress and apoptosis.

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Abstract

Azathioprine is a purine antimetabolite drug commonly used to treat inflammatory bowel disease (IBD). *In vivo* it is active after reaction with reduced glutathione (GSH) and conversion to mercaptopurine. Although this reaction may occur spontaneously, the presence of isoforms M and A of the enzyme glutathione-S-transferase (GST) may increase its speed. Indeed, in pediatric patients with IBD, deletion of GST-M1, which determines reduced enzymatic activity, was recently associated with reduced sensitivity to azathioprine and reduced production of azathioprine active metabolites. In addition to increase the activation of azathioprine to mercaptopurine, GSTs may contribute to azathioprine effects even by modulating GSH consumption, oxidative stress and apoptosis. Therefore, genetic polymorphisms in genes for GSTs may be useful to predict response to azathioprine even if more *in vitro* and clinical validation studies are needed.

INTRODUCTION

Azathioprine, the 1-methyl-4-nitroimidazol-5-yl derivative of mercaptopurine, is a purine antimetabolite drug commonly used to treat inflammatory bowel disease (IBD). Despite the introduction of effective biological treatments, such as antibodies against tumor necrosis factor- α (TNF- α), azathioprine is still a mainstay for maintenance therapy of severe IBD. Azathioprine is a prodrug and requires complex conversion to active metabolites (Figure 1). The first step in this conversion is the reaction of azathioprine with reduced glutathione (GSH), to yield the prodrug mercaptopurine and a nitroimidazole derivative/conjugate of GSH. Even though this reaction can occur spontaneously^[1] the presence of the enzyme glutathione-S-transferase (GST) may increase its speed,

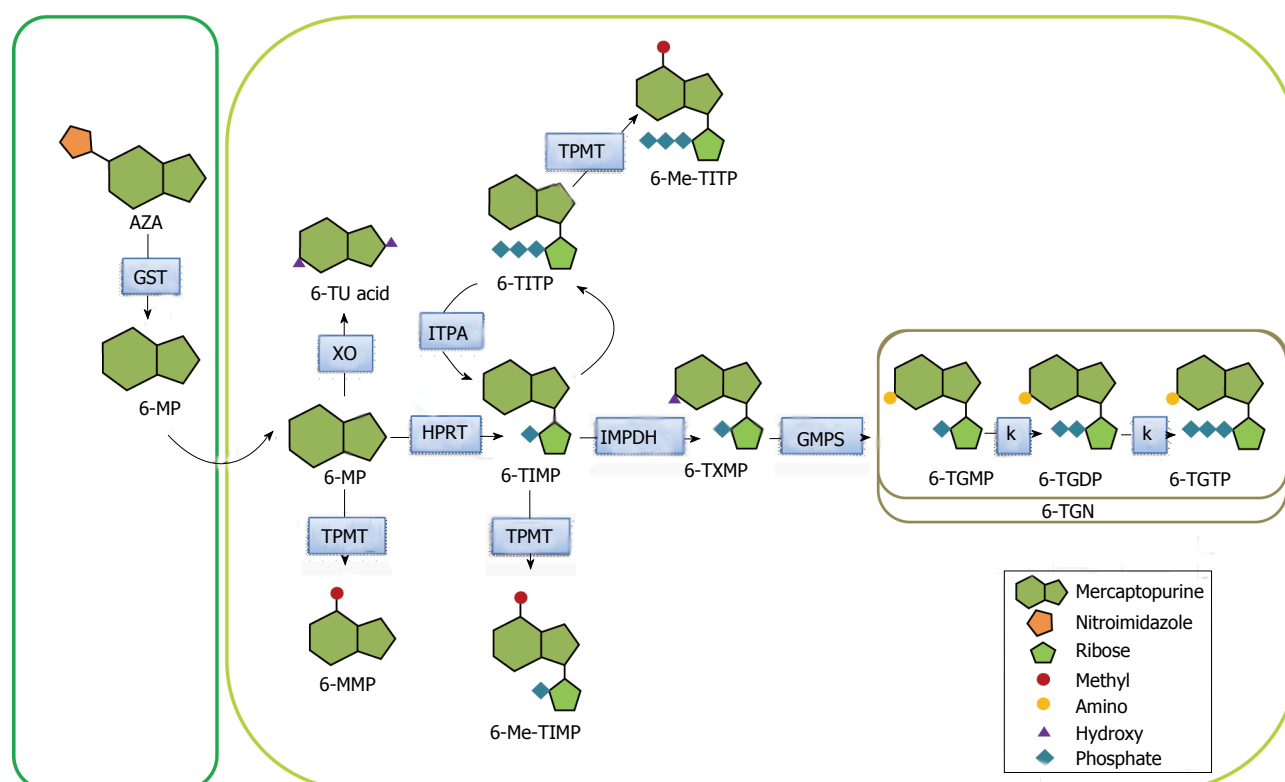


Figure 1 Metabolism of azathioprine and mercaptopurine. 6-Me-TIMP: 6-methyl-thioinosine-monophosphate; 6-Me-TITP: 6-methyl-thioinosine-triphosphate; 6-MMP: 6-methyl-mercaptopurine; 6-MP: Mercaptopurine; 6-TGDP: 6-thioguanine-diphosphate; 6-TGMP: 6-thioguanine-monophosphate; 6-TGN: 6-thioguanine nucleotide; 6-TGTP: 6-thioguanine-triphosphate; 6-TIMP: 6-thioinosine-monophosphate; 6-TITP: 6-thioinosine-triphosphate; 6-TU: 6-thiouric; 6-TXMP: 6-thioxanthosine-monophosphate; AZA: Azathioprine; GMPS: Guanosine-monophosphate-synthase; GST: glutathione-S-transferase; HPRT: Hypoxanthinephosphate-rybosil-transferase; IMPDH: Inosine-monophosphate-dehydrogenase; k: Kinase; TPMT: Thiopurine-S-methyl-transferase; XO: Xanthine-oxidase.

as discussed later. Even mercaptopurine needs metabolic conversion to thioguanine nucleotides (TGNs), catalyzed by the enzymes of the purine salvage pathway. Moreover, mercaptopurine is inactivated in the liver mainly by xanthine oxidase (XO), while in the extra hepatic tissues mercaptopurine catabolism involves predominantly genes that display genetically determined polymorphic activity, such as thiopurine-S-methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA). After oral administration, intact azathioprine is undetectable in blood because of extensive first pass metabolism^[2].

Mercaptopurine's cytotoxic effects are mainly related to incorporation of the active TGNs in the nucleic acids and to the consequent interference with the function of DNA processing enzymes and, to some extent, to inhibition of *de novo* purine synthesis, mainly operated by methylated precursors of TGNs.

While mercaptopurine pharmacokinetics and pharmacodynamics have been characterized extensively, the mechanism of conversion of azathioprine to mercaptopurine and its clinical implications for therapy personalization have not been completely elucidated.

EFFECTS OF GST POLYMORPHISMS ON AZATHIOPRINE EFFICACY AND METABOLISM IN PATIENTS WITH IBD

The hypothesis that patients with reduced levels of spe-

cific GST isoforms, due to genetic polymorphisms, may present decreased sensitivity to azathioprine because of a reduced enzymatic conversion of azathioprine to mercaptopurine, was tested recently by our team in young patients with IBD. The deletion of GST-M1, GST-T1 and the coding non synonymous single nucleotide polymorphism rs1695 in GST-P1 were tested. An initial study considered a cohort of 70 young patients (median age 16.2 years, 36 females) with IBD (41 Crohn's disease, 29 ulcerative colitis). Among these, 15 patients developed adverse events during treatment with azathioprine: in particular, there were three cases of bone marrow suppression, three cases of liver toxicity, seven cases of pancreatic toxicity, one case of neuropathy and one case of arthralgia; all these side effects resolved completely after the reduction or interruption of azathioprine administration: azathioprine was therefore considered the main determinant of the adverse effects. Interestingly, the candidate genetic association analysis in these patients revealed that frequency of GST-M1 deletion was significantly lower in patients that developed an adverse event in comparison to patients that tolerated azathioprine treatment with no adverse event (frequency of deletion respectively 26.7% *vs* 67.3%, $P = 0.0072$). Moreover, the incidence of mild lymphopenia (lymphocytes count under $1000/\text{mm}^3$), that was considered a marker of efficacy during azathioprine treatment, resulted associated with GST-M1 genotype: indeed, among patients tolerating azathioprine treatment

and with lymphopenia, frequency of the deletion was 28.6% in comparison to 72.9% among patients tolerant to azathioprine but that did not present this drug effect ($P = 0.032$)^[3]. Taken together, these results are in agreement with a model in which patients with GST-M1 deletion are less sensitive to the effects of azathioprine, putatively because of the contribution of this enzyme on the conversion of azathioprine to mercaptopurine. In a recent study, we evaluated the effects of GST polymorphisms on azathioprine metabolism in a cohort of 75 young patients (median age 15.2 years, 36 females) with IBD (46 Crohn's disease, 29 ulcerative colitis) tolerant to azathioprine therapy (taking azathioprine for more than 3 mo). Azathioprine metabolites were measured on samples collected from these patients using an high performance liquid chromatography assay (HPLC)^[4]: 150 measurements of azathioprine metabolites were collected. Patients with the deletion of GST-M1 tolerated a dose of azathioprine significantly higher in comparison to patients with normal GST-M1 (mean dose of azathioprine 2.1 mg/kg per day *vs* 1.8 mg/kg per day, $P = 0.022$). Moreover, the amount of active TGNs generated in patients with the deletion of GST-M1 was significantly decreased in comparison to patients with a normal genotype (mean amount of TGNs metabolites concentration for mg/kg of azathioprine: 252 pmol/ 8×10^8 erythrocytes *vs* 164 pmol/ 8×10^8 erythrocytes, $P = 0.0030$). Multivariate analysis confirmed that this effect was independent from that of other genes with a significant effect, such as TPMT, the main gene known to influence mercaptopurine metabolism^[5]. This study therefore supports a role of GST-M1 on azathioprine efficacy, mediated by an increased conversion of azathioprine to mercaptopurine. The reaction catalyzed by GST-M1 likely occurs after oral administration mainly in the intestine and the liver, modulating the amount of mercaptopurine and TGNs that are released in the main circulation.

These studies considered even the effect of GST-P1 and GST-T1 polymorphisms on azathioprine effects and metabolism but did not detect any significant association. The lack of association may be due to the tissue distribution of GST-P1 and GST-T1, which are not highly expressed in the liver, but even to the lack of specific activity of these enzymes toward the catalysis of the reaction of azathioprine with glutathione^[6]. Another study considered GST-M1 genetic polymorphisms as a candidate involved in azathioprine activation^[7]: this report considered 51 Asian patients with systemic lupus erythematosus (SLE) and the effect of polymorphisms in the ITPA, TPMT, GST-M1 and GST-T1 genes on the response to a low dose of azathioprine (0.97 mg/kg per day). Response to therapy, evaluated as a change in disease activity index, was associated with ITPA genetic polymorphism but not with the other ones. A clear interpretation of this paper results may be difficult because of the lack of data on azathioprine metabolites concentrations. However, the lack of an effect of GST-M1 on azathioprine efficacy in this study may be due to the very low dose of drug used. This study indicated that in Asian patients with SLE

the effect of ITPA might be predominant on those of TPMT and GST-M1 when azathioprine is used at very low doses; indeed, in this study even TPMT genetic polymorphism was not associated with azathioprine efficacy. It is known that in patients with Asian ancestry the frequency of variant TPMT is very low, while that of variant ITPA is increased in comparison to Caucasians^[8,9].

INVOLVEMENT OF GST IN AZATHIOPRINE MOLECULAR AND CELLULAR EFFECTS

GSTs are enzymes responsible for the inactivation of electrophilic substances, both endogenous and exogenous, by catalyzing their reaction with GSH. Human cytosolic GSTs are encoded by 17 genes and the proteins derived can be classified into 7 distinct classes based on their amino acid sequences. The most abundant GSTs in human cells are those of class P, M and A. GST-P1 is the principal isoform in most tissues, such as the small intestine and erythrocytes, but is not detectable in normal liver cells; on the other hand, GSTs M1, A1 and A2 are highly expressed in hepatocytes, while they are not expressed in erythrocytes. GST-T1 is expressed in the liver, intestine and erythrocytes even if its level of expression is lower compared to GSTs of the other classes^[6].

All the genes for these GST classes display common genetic polymorphisms that influence the activity of the enzyme in some individuals. For GST-M1 and T1, a common deletion is present in humans, so that about 50% and 20% of caucasians lack activity of these enzymes because of this genetic variant. In patients of African and Asian ancestry, frequency of GST-T1 deletion is higher than Caucasian, reaching around 50%, while frequency of GST-M1 deletion is similar^[10]. GST-P1 displays a common coding non-synonymous variant, an A-G transition at base 1578, resulting in the amino acid change I105V in the substrate binding site of the enzyme: the frequency of the variant genotype for this polymorphism in Caucasian and African is similar (13%-15%), while in Asian the percentage is lower (1%-2%)^[11,12].

Even expression of GST-A is modulated by genetic polymorphism: GST-A1 -69 C > T results in a reduced GST-A1 expression and related enzyme activity^[13,14]. The GST-A1 polymorphism shows different frequencies for the mutated genotype between Caucasian and African (respectively 17% and 11%) and Asian (1%). For GST-A2, a C-T transition at base 328, resulting in the amino acid change P110S in the electrophilic substrate binding site, occurs only in heterozygosis, at a frequency of 11% in Caucasian and 23% in Asian population^[15,16]. In the African population, the variant allele to date has not been found (Table 1). Genetic polymorphisms in GSTs, determining the interindividual variability in the activity of these important metabolic enzymes, have been related to the incidence of several pathologies, in particular oncological, and to altered sensitivity to medications, including azathioprine^[17-20].

Table 1 Glutathione-S-transferase polymorphisms and frequencies in different ethnic groups

Gene	Polymorphisms	Caucasian	African	Asian
GST-M1	Deletion	50%	50%	50%
GST-T1	Deletion	20%	50%	50%
GST-P1	rs1695 (A > G)	13% (GG)	15% (GG)	1%-2% (GG)
GST-A1	rs3957357 (C > T)	17% (TT)	11% (TT)	1% (TT)
GST-A2	rs2234951 (C > T)	11% (CT)	0% (CT)	23% (CT)

GST: Glutathione-S-transferase.

Enzymatic conversion

Azathioprine conversion to mercaptopurine can occur spontaneously^[1], even if in the presence of specific GST classes and at physiological pH values the reaction catalyzed by the enzyme may be prevalent.

Kaplowitz described an initial report on the enzymatic contribution on the conversion of azathioprine to mercaptopurine in rat liver homogenates. While at relatively high pH levels (*i.e.*, 8.0) the non-enzymatic reaction and the enzymatic one occur in similar proportions; at lower pH levels (*i.e.*, 6.5-7.4), closer to physiological values, the enzymatic reaction prevails^[21]. The same reaction has been demonstrated in homogenates of human livers: in these samples, mainly from kidney transplant donors, conversion of azathioprine to mercaptopurine was inhibited by treatment with furosemide, a GST inhibitor^[22]. Additional evidence obtained in animal models supports a significant contribution of the enzymatic conversion of azathioprine to mercaptopurine *in vivo*. Indeed, pretreatment of rats with probenecid, a GST inhibitor, determines a greater proportion of unmetabolized azathioprine in the liver and less hepatic GSH depletion. Bilirubin is also a GST inhibitor and, in a model of hyperbilirubinemic rat (Gunn rat), less hepatic GSH depletion was found during exposure to azathioprine^[23]. These observations indicate that the conversion of azathioprine to mercaptopurine *in vivo* is mediated enzymatically by the GSTs. After oral administration of azathioprine this reaction likely occurs mostly in the liver: indeed after oral administration azathioprine is undetectable in blood, while mercaptopurine appears after either oral or *iv* azathioprine administration^[2]. In addition it has been shown that after *ip* injection of azathioprine in rats, GSH was depleted rapidly in hepatocytes but not in other tissues (*i.e.*, erythrocytes, kidneys and intestine) indicating that after administration of azathioprine, the hepatic contribution to total GSH consumption may be predominant^[24].

Eklund *et al.*^[6] have shown that among 14 GSTs tested, GST-A1, GST-A2 and GST-M1 displayed the highest activity on the catalysis of azathioprine to mercaptopurine; these enzymes are all highly expressed in human hepatocytes and therefore in these cells the uncatalyzed reaction of azathioprine with GSH was estimated to be less than 1% of the GST-catalyzed biotransformation. Interestingly, GST-M1 and GST-A display genetically determined variable expression levels. These differences in GST activity may result in interindividual differences in

the conversion of azathioprine to mercaptopurine. The authors suggest that individuals with high levels of GST could be particularly sensitive to azathioprine and potentially more prone to adverse effects during treatment with azathioprine, because of both increased concentrations of free mercaptopurine and of a more pronounced GSH depletion.

Oxidative damage

Considering the intracellular enzymes involved in thiopurines' metabolism, it is reasonable to suggest that these agents are able to induce oxidative stress. Indeed, it has been demonstrated that thiopurines can generate directly reactive oxygen species (ROS) in cells exposed to ultra violet light^[25,26]. Moreover, regarding azathioprine, some evidence suggest an indirect ability to induce oxidative stress, mediated primarily by GSH consumption during the metabolic conversion of azathioprine to mercaptopurine^[27,28]. GSTs, influencing the reaction of azathioprine with GSH, may influence therefore the cellular effects of azathioprine mediated by oxidative damage. Moreover, even metabolism of thiopurines by XO may generate ROS, as assessed on primary cultures of rat hepatocytes^[28]. XO, which metabolizes mercaptopurine, converting it to thiouric acid, is a well know producer of ROS, such as superoxide anion^[29], whose accumulation could worsen the oxidative stress induced by GSH depletion. Allopurinol, a XO inhibitor, has been shown to restore response to thiopurines in patients with IBD unresponsive to thiopurines and with unfavorable metabolic ratio, increasing the concentration of active TGNs and decreasing those of the methylated nucleotides, likely because of inhibition of TPMT^[30]. Cellular redox balance is largely determined by GSH. Depletion of such cellular antioxidant defenses allows the accumulation of significant amounts of ROS, as demonstrated in several systems^[31,32], which, in turn, have been suggested to act as a signal for apoptosis induction^[33]. Regarding azathioprine, exposure to this medication *in vitro* induces a rapid depletion of GSH in hepatocytes before any loss of viability. Addition of exogenous GSH or N-acetylcysteine protected against cell death. Lee *et al.*^[27] suggested that oxidative stress induced by GSH depletion is able to induce mitochondrial damage, opening of mitochondrial permeability transition pore (MPTP) and rapid consumption of adenosine triphosphate. Ultrastructural analysis showed occurrence of necrosis after azathioprine exposure. The fact that, under the same experimental conditions, mercaptopurine was not able to reduce hepatocyte viability, allows suggesting that the activating steps triggered by GST and the associated GSH depletion could be a crucial step in azathioprine cytotoxicity *in vitro*.

Among the many secondary effects attributed to them, a role for ROS in mediating an anti-proliferative effect has been demonstrated. Indeed, the cellular redox balance fluctuates during cell cycle, so that the redox state modulates cell cycle progression from one phase to the next^[34]. In this scenario, significantly higher GSH content

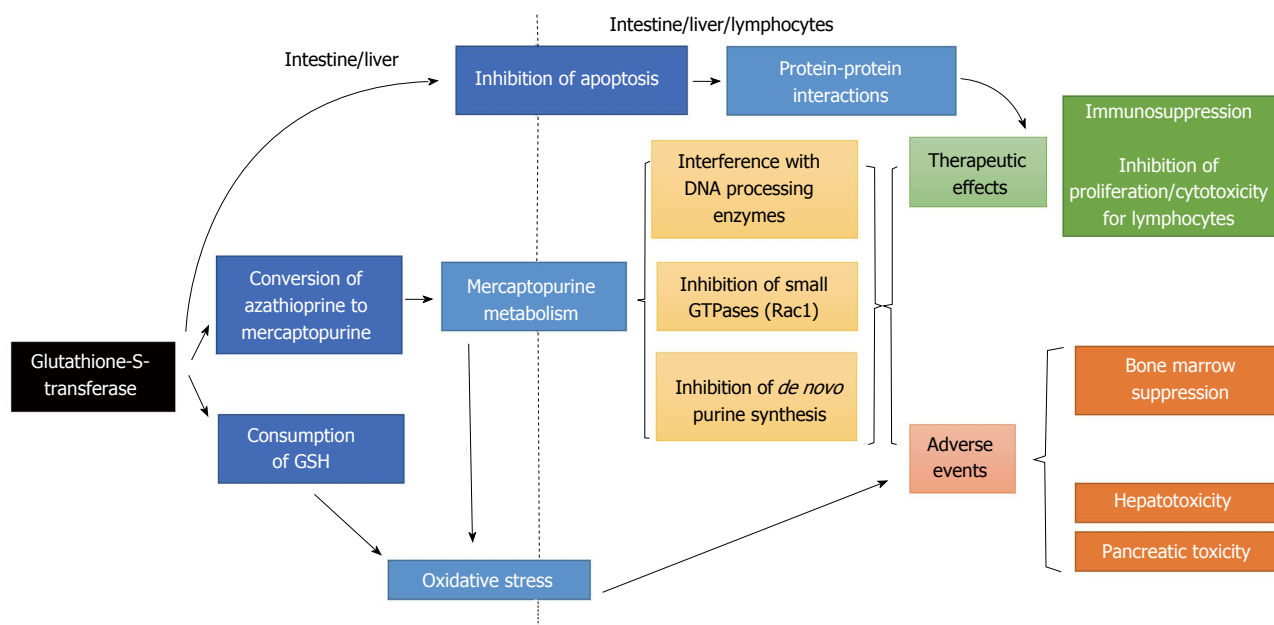


Figure 2 Schematic representation of different glutathione-S-transferase catalyzed phenomena (blue boxes) that even through intermediate events (light blue) bring or contribute to the canonical mechanisms of action (yellow), determining the therapeutic effects (green) and/or adverse events (orange). GSH: Reduced glutathione.

in the G₂ and M phases compared with G₁ were found^[35]. Hence, it is reasonable to hypothesize a role for ROS in affecting the anti-proliferative effect of thiopurines, which are cell cycle phase specific agents and especially azathioprine, which consumes GSH during its conversion to mercaptopurine.

Azathioprine half-life is very short and its therapeutic effects on lymphocytes are likely due to the metabolites produced after first pass metabolism in the intestinal and liver cells. However, azathioprine cytotoxic effects due to GSH consumption are likely to play an important role on the development of cytotoxicity in intestinal and liver cells, supporting a role in the induction of adverse events in these organ systems. However, the fact that on hepatocytes azathioprine is able to modulate a necrotic cell death through a cyclosporine-A sensitive MPTP opening, rise some doubts on the actual role of oxidative stress^[27]. Studies are still required to demonstrate if ROS could represent an alternative mechanism of cytotoxicity induced by azathioprine and its clinical relevance.

Modulation of apoptosis

Azathioprine and mercaptopurine induce apoptosis in activated lymphocytes and these effects should be crucial in determining the efficacy of these medications as immunomodulators in young patients with IBD^[36]. GSTs have been shown to modulate apoptosis and the incidence of lymphopenia during treatment of IBD patients and therefore modulation of apoptosis by GSTs may be of significant relevance as a mechanism for the role of these proteins on azathioprine effects.

GST-P1 was the first isoenzyme found to play a role in signaling pathways that control cell survival, and so far is the most important one. This regulation role is

achieved independently from the well-known conjugating activity and occurs through a physical protein-protein interaction with c-Jun N-terminal Kinase (JNK), a member of the mitogen-activated protein kinases (MAPK), with the consequent inhibition of the downstream JNK-induced apoptotic pathway. GST-M1 plays a similar role of negative regulator by physically sequestering the apoptosis-signaling regulating kinase 1 (ASK-1), a MAPK kinase that activates both JNK and stress responsive p-38 kinase (another MAPK)^[37]. Stress triggers, such as heat shock or the pro-inflammatory cytokines TNF- α and interleukin 1- β , promotes the dissociation of GST-M1 from ASK1, resulting in the activation of ASK-1 and the phosphorylation-dependent activation of p38^[38]. The final cell fate (proliferation or apoptosis) depends on the strength and duration of the cellular stress.

GST-A1 has been found to suppress activation of JNK signaling by a pro-inflammatory cytokine and oxidative stress in Caco-2 cells, suggesting also a protective role for GST-A1 in JNK-associated apoptosis^[39].

The expression of GSTs of the M and A classes has been reported to drop under apoptotic conditions and their overexpression was able to block apoptosis in rat hepatocytes^[40]. GST-M1 has been reported to bind to ASK1 and inhibit apoptosis^[37].

Matsumaru *et al.*^[41] reported that depletion of cytosolic GSH could sensitize murine hepatocytes to apoptosis induced by TNF- α ^[41,42]; clinical studies have shown that TNF- α protein and mRNA levels are elevated in serum, intestinal tissue and stools of active IBD, in correlation with disease activity^[43]. Therefore, even depletion of GSH catalyzed by GST could make cells of patients with IBD particularly sensitive to the cytotoxic effects of thiopurines, potentially leading to an increased incidence

of adverse events.

CONCLUSION

It would be important that other studies validate the observation of the increased conversion of azathioprine to mercaptopurine in patients with normal GST-M1, resulting in increased sensitivity to the medication in patients with IBD, both clinically and using *in vitro* experiments. Moreover, since GSTs of the A class are highly expressed in the liver, have catalytic activity on the conversion of azathioprine to mercaptopurine and display genetically determined polymorphic activity, it would be important to evaluate the role even of variation in genes for the A class of GST on azathioprine pharmacokinetics and efficacy.

Further insights on the role of genetic polymorphisms of GST and other enzymes on azathioprine pharmacogenetics could come from the use of innovative and more sensitive methods for the measurement of azathioprine metabolites. Indeed, most of the research published so far, including the papers mentioned in this report, have characterized thiopurine metabolites in erythrocytes from patients with IBD, using HPLC methods that group all thionucleosides as TGNs or methylated nucleotides^[44], without distinguishing the degree of phosphorylation, which may be of relevance for thiopurines' cellular effects^[45]. Given the complexity of thiopurines' metabolism, methods with increased sensitivity, allowing to assess the nucleotides degree of phosphorylation and potentially the identification of additional relevant species, such as those based on mass spectrometry are of great interest^[46]. Moreover, these methods with increased sensitivity allow the use of very small volumes of patients' samples and this is particularly relevant for pediatric patients^[47,48].

The increasing complexity^[49] of thiopurines' pharmacogenetics has been consolidating: while TPMT is the strongest determinant of variability in the pharmacokinetics of these medications^[50], currently used in several clinical protocols to adjust treatment with thiopurines, even other genes, such as ITPA, have been shown to be of relevance^[51]. Based on the clinical and *in vitro* evidence highlighted in this paper (Figure 2), it seems that for azathioprine even GST-M1 genetic polymorphism could enter in a useful multi-locus genotype to predict patients' response to this medication. However, the association of GST-M1 with azathioprine efficacy in patients with IBD still needs to be supported mechanistically by *in vitro* studies and validated by adequately sized prospective clinical trials.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Anemia in inflammatory bowel disease: A neglected issue with relevant effects

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Abstract

Anemia, a common complication associated with inflammatory bowel disease (IBD), is frequently overlooked in the management of IBD patients. Unfortunately, it represents one of the major causes of both decreased quality of life and increased hospital admissions among this population. Anemia in IBD is pathogenically complex, with several factors contributing to its development. While iron deficiency is the most common cause, vitamin B₁₂ and folic acid deficiencies, along with the effects of pro-inflammatory cytokines, hemolysis, drug therapies, and myelosuppression, have also been identified as the underlying etiology in a number of patients. Each of these etiological factors thus needs to be identified and corrected in order to effectively manage anemia in IBD. Because the diagnosis of anemia in IBD often presents a challenge, combinations of several hematimetric and biochemical parameters should be used. Recent studies underscore the importance of determining the ferritin index and hepcidin levels in order to distinguish between iron deficiency anemia, anemia due to chronic disease, or mixed anemia in IBD patients. With regard to treatment, the newly introduced intravenous iron formulations have several advantages over orally-admin-

istered iron compounds in treating iron deficiency in IBD. In special situations, erythropoietin supplementation and biological therapies should be considered. In conclusion, the management of anemia is a complex aspect of treating IBD patients, one that significantly influences the prognosis of the disease. As a consequence, its correction should be considered a specific, first-line therapeutic goal in the management of these patients.

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Key words: Anemia; Inflammatory bowel disease; Iron deficiency; Anemia of chronic disease; Erythropoietin

Core tip: Anemia represents one of the major causes of both decreased quality of life and increased hospital admissions among inflammatory bowel disease (IBD) patients. This paper analyses the complex etiological and pathophysiological mechanisms underlying anemia in IBD, including iron and micronutrients deficiency, effects of proinflammatory mediators and bone marrow insufficiency secondary to the disease by itself and IBD therapy. By a comprehensive review of the current diagnostic and therapeutic evidences on anemia in IBD, an state-of-the-art approach will be provided to effectively manage this challenging and common condition.

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INTRODUCTION

Anemia, a frequent systemic complication in patients

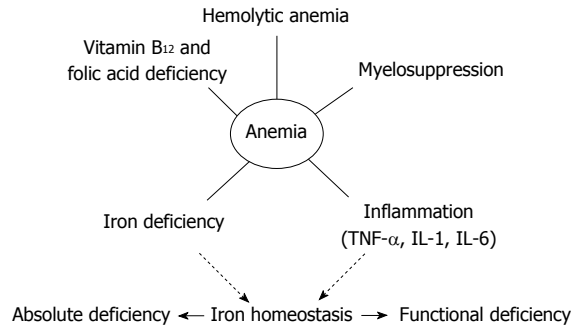


Figure 1 Pathogenesis of multifactorial anemia associated to inflammatory bowel disease. TNF- α : Tumour necrosis factor- α ; IL: Interleukin.

with inflammatory bowel disease (IBD), has a complex and multifactorial pathogenesis (Figure 1). It is considered a prototype of a combination of iron deficiency (IDA) and anemia of chronic disease (ACD), which is caused by the negative effects of an activated immune system at different levels of erythropoiesis^[1,2]. Besides IDA and ACD, metabolic disturbances, vitamin deficiencies, and various drug therapies commonly used in IBD can aggravate anemia in IBD patients^[3]. The study of anemia in these patients thus requires a specific diagnostic and therapeutic approach.

It is important to highlight that anemia has a significant impact on the disease and is one of the most frequent comorbid conditions associated with mortality in IBD patients^[4]. In addition, it also has a relevant effect on health related quality of life (QoL) and ability to work^[5,6]. The fact that it is also a common cause of hospitalization and delay of discharge^[7] only serves to underscore the need for prompt diagnosis and management of this condition.

Although the correction of anemia in IBD patients can improve the QoL and the quality of patient management, the specific diagnosis and treatment of anemia is often a low priority for gastroenterologists and has thus received little attention. A recent study showed that further diagnostic tests were undertaken in only one-third of patients with proven anemia and that 54.3% of patients diagnosed with IDA receive no iron supplements^[8,9].

This article reviews current data on the diagnosis and treatment of anemia in IBD patients. A search was conducted in the PubMed, Cochrane, MEDLINE, and Scopus libraries with the following individual and combined key words: Crohn's disease, ulcerative colitis, anemia, iron deficiency anemia, anemia of chronic disease, vitamin B₁₂ deficiency, folic acid deficiency, myelodysplastic syndrome, refractory anemia, iron supplementation, intravenous iron therapy, erythropoietin, and inflammatory bowel disease. References cited in the articles retrieved were also searched in order to identify other potential sources of information. The results were limited to human studies available in English.

PREVALENCE OF ANEMIA IN INFLAMMATORY BOWEL DISEASE

The prevalence of anemia in IBD is markedly variable, ranging from 6% to 74% in two systematic reviews^[10,11]. The more recent review calculated a mean prevalence of 17% (16% in outpatients and 68% in hospitalized patients), with anemia occurring more frequently in patients with Crohn's disease (CD) than in those with ulcerative colitis (UC)^[3,12]. This variability in the prevalence of anemia depends on different factors. First, the definition of anemia is not homogenous in the various studies reviewed. In fact, because the widely accepted World Health Organization criterion for the diagnosis of anemia [hemoglobin (Hb) below 13 g/dL in men or 12 g/dL in non-pregnant women]^[13] has been questioned due to racial differences, environmental conditions, and eating habits^[14,15], its use cannot completely reflect the real prevalence of anemia in different IBD populations. Furthermore, estimations of the prevalence of anemia often depend on the specific groups of patients studied (for example, hospitalized patients *vs* outpatients). In this sense, a study from a Swedish cohort showed that the prevalence of anemia in hospitalized UC and CD patients was higher than among outpatient populations (5% *vs* 35% and 9% *vs* 50%, respectively)^[16]. Furthermore, it is important to note that anemia has been poorly studied in pediatric IBD patients. One recent epidemiological study showed that while the prevalence of anemia was 72% at the time of diagnosis, the proportion of severely anemic pediatric patients decreased from 34% to 9% while the number of patients with mild anemia doubled after 1 year of follow-up^[17]. Finally, because Hb levels form part of a widely used disease activity index, the presence of anemia correlates directly with disease activity, which means that the prevalence of anemia may change throughout the natural history of the disease. In fact, the prevalence of mild and moderate anemia in IBD has decreased over time, reflecting improved treatment and management of the disease. However, the prevalence of severe anemia in IBD patients over the last 10 years has not decreased in the same manner^[18].

PATHOGENESIS OF ANEMIA IN INFLAMMATORY BOWEL DISEASE

Iron deficiency anemia

Iron deficiency is the most common cause of anemia in IBD patients, with a reported prevalence of up to 90%^[11]. Iron deficiency may be related to "absolute iron deficiency" due to low dietary intake and blood loss from ulcerated intestinal mucosa (especially in UC patients) along with reduced iron absorption (especially in CD localized in the upper GI tract), or it may be related to "functional iron deficiency."

Iron is an essential mineral for the function of all

body cells and is absorbed at the apical surface of enterocytes to be transported by ferroportin across the basolateral surface of the enterocyte into the circulation^[19]. In the maintenance of iron homeostasis, the peptide hormone hepcidin is a master regulator that is produced in response to iron overload or upon induction by pro-inflammatory stimuli such as lipopolysaccharide or interleukin (IL)-6. In fact, inflammatory conditions can interfere with iron absorption by causing an increase in hepcidin that inhibits ferroportin activity^[20], leading to its internalization and degradation^[21]. The inhibition of ferroportin activity blocks the transfer of absorbed iron from the enterocyte into the circulation and causes iron retention in the macrophages and monocyte cells^[22]. In addition, during inflammation other events contribute to the retention of iron in these cells, including the inhibition of ferroportin transcription by pro and anti-inflammatory cytokine action and a reduction in the half-life of erythrocytes due to oxidative stress and lipid-peroxidation, with iron recycling through erythrophagocytosis^[23]. These mechanisms all lead to “functional iron deficiency,” which means that despite an abundance of iron in the body, it is not available for erythropoiesis.

Anemia of chronic disease

The exact prevalence of ACD in IBD patients is unknown^[24], with its etiology being ascribed to altered erythropoiesis at different levels^[25]. Firstly, chronic inflammation can decrease erythropoiesis by direct action of interferon (IFN)- γ , IFN- α , tumor necrosis factor (TNF)- α , and IL-1 in the bone marrow to exert pro-apoptotic effects on erythroid burst-forming units (BFU-E) and colony-forming units (CFU-E)^[26]. Moreover, IL-1, IL-6, TNF- α , and hepcidin may decrease erythropoietin (EPO) synthesis and impair its biological activity^[1,27]. In fact, EPO levels in ACD have been found to be inadequate in some chronic disease and IBD patients^[11,28,29]. Low EPO production is due to direct inhibition of the activity of the promoter of the EPO gene by IL-1 and TNF- α , which in turn inhibits the synthesis of EPO in the kidney and acts indirectly on EPO-producer cells through cytokine-induced toxic radicals^[30]. Impairment of the biological activity of EPO means that much higher amounts of EPO are needed to restore the formation of CFU-E in the bone marrow. Cytokines can also interfere with the signaling process mediated by the interaction of EPO and its receptor and can down-regulate EPO receptors on erythroid progenitor cells^[26], thus producing cell resistance to EPO activity. Finally, the limited availability of iron for heme biosynthesis induced by “functional” or “absolute” iron deficiency and the inhibition of iron uptake into erythroid progenitors due to the blocking of the transferrin receptor by α 1-antitrypsin (an acute phase protein) negatively affect the biological functions of EPO along with cell growth and differentiation^[1].

Other types of anemia

Vitamin B₁₂ deficiency has been observed in 48% of

CD patients and in 5% of UC patients^[31] while folic acid deficiency has been noted in 67% of CD patients and in 30%-40% of UC patients^[31-33]. These types of deficiencies depend on low dietary intake as well as increased turnover of epithelial cells due to chronic inflammation in the intestinal mucosa and a reduced absorption in the intestinal tract^[34-36]. In CD, several factors influence these deficiencies, including the inflammatory involvement of ileal mucosa, the presence of fistulas, secondary bacterial overgrowth with direct consumption of vitamin B₁₂, and extensive surgical resections in small bowel segments with impaired absorption^[37]. Deficiencies in patients with UC derive from proctocolectomy and ileo-pouch anastomosis, with the prevalence of vitamin B₁₂ deficiency being affected more by surgical changes leading to impaired function of ileal receptors, reduced intestinal transit time, and secondary bacterial overgrowth than on the length of the ileal segment resected^[38].

Autoimmune hemolytic anemia (AIHA) is a rare type of anemia observed in UC patients. It can be due either to the development of antibodies with cross-reactivity with erythrocytes^[39] or to the hemolytic effect of sulfasalazine in patients with glucose-6-phosphate dehydrogenase deficiency^[40]. This association was first described in 1955 by Lorber *et al*^[41] with the most recent studies calculating that the prevalence of AIHA in UC patients is between 0.2%-1.7%, as indicated by a positive Coombs test result in 1.8% of patients studied^[40]. AIHA can occur before, after, or at the moment of diagnosing UC. Even when the potential relationship between disease activity and the occurrence of AIHA is not clear, a correlation with the extension of the disease has been demonstrated in several reports, which show a prevalence of AIHA of up to 28% in patients with extensive colitis^[40].

Anemia can also represent a late manifestation of myelosuppression in IBD patients due to several factors. Firstly, myelosuppression can be associated with myelodysplastic syndrome (MDS), with ineffective erythropoiesis and a risk of progression to acute myeloid leukemia. Some studies have shown a frequent predominance of MDS in CD with colorectal involvement; however, it should be noted that the prognosis of IBD with concomitant MSD is determined by the MSD itself^[42]. The prevalence of MDS in IBD patients has been estimated to be 0.17%, with a higher incidence in IBD patients than in the general population (170/100000 IBD patients/year *vs* 20-30/100000 of the general population over the age of 70/year)^[43]. This is probably due to a undetermined common pathogenetic mechanism, the long-term use of immunosuppressive drugs, or chromosomal abnormalities in bone marrow cells that have been observed in 67% of patients with concomitant IBD and MSD^[44,45]. These can induce the development of colitogenic monocytes, producing a large number of pro-inflammatory cytokines resistant to apoptosis upon stimulation with microbial antigens. Indeed, one of the first hypotheses about this association regarded IBD to be an extra-hematologic manifestation of MSD with a

Table 1 Laboratory findings in anemia of inflammatory bowel disease patients

Biomarkers	IDA	ACD	Mixed anemia
MCV (fL)	< 80	Normal or reduced	Normal or reduced
MCH (pg)	< 27	Normal	Normal
CHr (pg)	< 28	Normal	Normal
C-RP (mg/dL)	Normal	> 5	> 5
ferritin (ng/mL)	< 30	> 100	30-100
TfS (%)	< 20	< 20	< 20
Ferritin index	> 3.2	< 11	> 2
sTfR	Increased	Normal	Normal or increased
Hepcidin (nmol/L)	Reduced	Increased (> 4)	Increased (> 4)

MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; CHr: Reticulocyte hemoglobin content; C-RP: C-reactive protein; TfS: Transferrin saturation; sTfR: Soluble transferrin receptor; IDA: Iron deficiency anemia; ACD: Anemia of chronic disease.

vasculitic process at the level of the mesenteric arteries^[46]. Alternatively, myelosuppression may represent a complication of severe UC with the development of a systemic inflammatory response syndrome, or even been a side effect of immunosuppressive drugs. There is an increasing concern about therapy-induced leukemias and myelodysplastic syndromes in patients treated with thiopurines, which are extensively used as immunosuppressants in IBD, particularly for maintenance therapy^[47]. Data from a large French cohort of patients (19486) with inflammatory bowel disease identified a relative risk of developing lymphoproliferative disorders as 5.2 for patients who were treated with thiopurines compared to those who were not^[48].

Finally, additional gastrointestinal diseases that do not usually cause bleeding should be also considered in case of iron deficiency anemia in those IBD patients who maintain disease into remission, including colon or gastric cancer or polyps, peptic ulcer, hiatal hernia with linear erosions, atrophic or *Helicobacter pylori*-associated gastritis, and celiac disease^[49,50].

DIAGNOSIS OF ANEMIA IN INFLAMMATORY BOWEL DISEASE

Basic laboratory screening for anemia in IBD should consist of hemoglobin and full blood counts (including reticulocytes to differentiate between regenerative or hypo-regenerative anemia), with a determination of erythrocyte mean corpuscular volume (MCV) to distinguish between microcytic, normocytic, and macrocytic anemia as well as a determination of mean corpuscular Hb (MCH) and reticulocyte Hb content (CHr), if available. Moreover, assessments of both the level of inflammation by means of C-reactive protein (CRP) and of iron status are required. There is no single biomarker to diagnose iron deficiency in IBD; a combination of different biomarkers is needed. In most cases, total store of body iron with serum-ferritin (or ferritin) and the iron available in the bone marrow with transferrin saturation (TfS) is sufficient to differentiate between IDA and

ACD^[51]. However, many of the laboratory measures of iron status may be unreliable in IBD patients because the inflammation influences all parameters of iron metabolism to produce “functional iron deficiency”^[52,53]. For this reason, in some cases it is essential to use other, more specific biomarkers of iron status to allow for the differentiation between predominantly IDA, predominantly ACD, and ACD combined with iron deficiency in order to provide appropriate, more effective treatment^[54] (Table 1). Further testing for causes of anemia in IBD may include tests for vitamin B₁₂, folic acid (especially erythrocyte levels, which, when available, represent the best stable marker of folic acid deficiency), haptoglobin, lactate dehydrogenase, indirect bilirubin (with Coombs test if hemolytic anemia is confirmed), and serum creatinine in order to rule out potential hemolysis or renal failure, which in itself can cause macrocytic or normocytic anemia^[51]. It should be noted that if the origin of anemia is not obvious, IBD patients should be tested for MDS, especially if normocytic and hypo-regenerative anemia are both present, carrying out a bone marrow study in selected patients.

Once a diagnosis of IBD has been established, patients in clinical remission should be screened for anemia at least every 6 to 12 mo, whereas patients with active disease should be tested every 3 mo or at even shorter intervals, depending upon their iron status^[55].

Iron deficiency anemia

Patients are considered to suffer from IDA when they present with low Hb (men < 13 g/dL, non-pregnant women < 12 g/dL), TfS < 20%, and ferritin concentrations < 30 ng/mL without any biochemical or clinical signs of inflammation. A low MCH (< 27 pg) or even better a low CHr (< 28 pg) rather than MCV (< 80 fL) have become the most important red cell markers for detecting iron deficiency in circulating red blood cells. Although MCV is a reliable and widely available measurement, it tends to be a relatively late indicator in patients who are not actively bleeding^[56]. A normal Hb level does not rule out iron deficiency and with an MCH in the lower limit of normal (normal range: 28-35 pg) or an increased red cell distribution width (RDW, normal range: 11-15), one can suspect the presence of mild iron deficiency without anemia^[57]. Although the main laboratory marker for iron deficiency with or without anemia is a low ferritin level (< 30 ng/mL) in the absence of inflammation, in the presence of inflammation a normal ferritin level (as an acute phase reactant) does not rule out iron deficiency; therefore, TfS should also be measured. “Functional iron deficiency” in inflammatory conditions should be defined by low TfS (< 20%) and normal ferritin concentration (> 100 ng/mL), whereas low TfS (< 20%) and intermediate ferritin values (30-100 ng/mL) suggest “absolute iron deficiency”^[57]. Some authors suggest a cut-off value of TfS < 16% combined with low iron value for the diagnosis of iron deficiency^[51]. Iron deficiency can also be defined by a ferritin index > 3.2 (> 2 if CRP > 5 mg/L). The ferritin index, which reflects

the iron supply for erythropoiesis, is calculated as the ratio between the soluble transferrin receptor (sTfR) and the log of ferritin^[58]. The sTfR is a truncated fragment of the membrane receptor and its levels increase when the availability of iron for erythropoiesis is low, as occurs in IDA. CHr, which measures the Hb content of reticulocytes, reflects the direct measurement of available iron for erythropoiesis and is useful for differentiating IDA from ACD. In particular, CHr has a high sensitivity and specificity for diagnosing iron deficiency and is less affected by inflammation than TfS and ferritin, but no data are available for its use in IBD^[58].

Anemia of chronic disease

Patients are considered to suffer predominantly from ACD when they present evidence of inflammation (with increased levels of serum CRP and clinical signs), an Hb concentration < 13 g/dL for men and < 12 g/dL for non-pregnant women, and a low TfS < 20%, but normal or increased ferritin concentrations > 100 ng/mL. In the presence of intermediate ferritin concentrations (30-100 ng/mL), a diagnosis of ACD combined with “absolute iron deficiency” is confirmed if the ferritin index has a value < 2 with normal CHr^[54,58-60]. Still, some cases may require supplementary testing for the differential diagnosis between IDA and ACD. It has recently been shown that hepcidin levels may replace the ferritin index for the confirmation of combined IDA and ACD if the hepcidin levels are > 4 nmol/L with a CHr < 28 pg^[61]. In fact, hepcidin levels have been found to be significantly higher in IBD patients compared with healthy controls, with a significant correlation with ferritin levels, CRP, and disease activity, whereas those of prohepcidin were observed to be significantly lower^[62]. In addition, although other hematological indices may help in the diagnosis of iron deficiency in ACD, many of them are only available in specific hematology analyzers and their precise clinical usefulness has yet to be determined. In a recent study carried out with a Beckman-Coulter LH 780, high values of RDW and low values of blood cell Size Factor were the best markers for the diagnosis of IDA, whereas both Reticulocyte Distribution Width-Coefficient of Variation (RDWR-CV) and Reticulocyte Distribution Width-Standard Deviation (RDWR-SD) were significantly correlated to disease activity and CRP levels^[63].

TREATMENT OF ANEMIA IN INFLAMMATORY BOWEL DISEASE

Iron supplementation

Iron supplementation should be considered in every patient presenting iron deficiency with or without anemia. In patients with mild to moderate anemia (Hb \geq 10 g/dL), the administration of oral iron at optimal low doses of 60-120 mg/d is the conventional approach recommended by the Centers for Disease Control and Prevention^[9,64]. Oral iron compounds are mostly available as inorganic ferrous salts, such as ferrous fumarate, ferrous

sulphate, and ferrous gluconate containing 33%, 20%, and 12% of elemental iron, respectively. A single tablet of most of these ferrous salt preparations provides a sufficient dose for the treatment of iron deficiency^[65,66]. In fact, there is no evidence to support the administration of high doses of iron in comparative trials^[65-67]; on the contrary, excessive doses may actually decrease tolerance and compliance while increasing gastrointestinal side effects, with a discontinuation of iron treatment in 20% of patients with or without IBD^[68]. Nevertheless, there are several drawbacks associated with oral iron therapy that must be taken into account. In addition to the generally low bioavailability of oral iron, intestinal absorption is further compromised in IBD patients due to an inflammation-driven blockade caused by increased hepcidin levels. For this reason, in patients with active inflammation and combined ACD/iron deficiency, intravenous administration of iron may be preferable to oral iron therapy. Moreover, when achieved, the therapeutic effect of oral iron supplementation is slow, requiring two to three weeks to obtain increased Hb concentrations and up to two months to achieve normal values. At least six months are needed to replenish iron stores completely^[69]. Moreover, non-absorbed iron salts can be toxic to the intestinal mucosa and oral iron has been shown to increase intestinal inflammation and possibly colon carcinogenesis in animal models through the production of reactive oxygen species that mediate intestinal damage and the alteration of the intestinal bacterial milieu in rodents^[70-75].

Intravenous iron therapy is more effective, has a higher response rate, and is better tolerated by patients, with a lower discontinuation rate due to adverse events than oral iron supplementations in IBD patients, as demonstrated in a recent systematic review with meta-analysis^[76]. However, it is important to highlight that of 757 articles identified, only three industry-funded articles met the inclusion criteria for this systematic review^[69,77-83]. Nevertheless, intravenous iron therapy should be considered in patients with severe anemia (< 10 g/dL), with intolerance or inadequate response to oral iron, or with concomitant erythropoietin treatment and/or presence of active IBD. It should be noted that the new intravenous iron formulations (iron carboxymaltose, iron ferumoxytol, and iron isomaltoside) reduce both the risk of free iron reactions as well as that of immunogenicity without the need for administering a test dose before starting treatment. Treatment duration is also reduced because the new formulations are safer at higher doses than traditional intravenous iron formulations (iron sucrose, ferric gluconate, and low molecular weight iron dextran). Iron carboxymaltose is the only new intravenous iron formulation approved for use in Europe that has been studied in IBD patients. Its superiority at higher standardized doses over individually calculated doses of iron sucrose has been demonstrated along with its efficacy in reducing anemia recurrence as compared to a placebo^[84-86]. After 12 wk of follow-up,

ferric carboxymaltose led to higher response rates (66.1% *vs* 54.1%), a higher proportion of non-anemic patients (72.8% *vs* 61.8%), and better treatment adherence (92.5% *vs* 79.1%) than iron sucrose, with no difference in treatment-related adverse events (13.9% *vs* 11.3%). With regard to side effects, in the ferric carboxymaltose group there were more skin and subcutaneous tissue disorders (rash, dermatitis, pruritus) and more cases of hypophosphatemia, but fewer infusion site reactions than in the iron sucrose group. The superiority of high-dose intravenous iron supplementation in IBD probably depends on the iron overload produced in the macrophages of the reticulo-endothelial system. This induces an overexpression of ferroportin, which may, in turn, by-pass the hepcidin block in ACD^[57]. Recently, an alternative dosage scheme to the traditional Ganzoni formula has been presented for ferric carboxymaltose treatment. In the new protocol, for a baseline Hb > 10 g/dL, the dose is 1.0 g for patients with a body weight < 70 kg and 1.5 g for patients > 70 kg; the corresponding total doses for serum Hb ≤ 10 g/dL are 1.5 g and 2.0 g^[84]. Phase 3 clinical trials are currently underway to evaluate the use of ferumoxytol in patients with iron deficiency anemia, including a subgroup with IBD (ClinicalTrials.org identifier: NCT01114139, NCT01114217 and NCT01114204). However, ferumoxytol may be problematic in IBD patients because it can interfere with MRI signals due to the paramagnetic nature of its iron core^[19]. In addition, a Phase 3 clinical trial of iron isomaltoside (NCT01017614) and a Phase 4 study of iron sucrose (NCT01067547) are currently being carried out on IBD patients with IDA. All treatments being tested strive to achieve ferritin concentrations > 100 µg/L, measured as early as 8 wk after intravenous iron treatment to obtain a reliable result^[55]. Considering that the recurrence of iron deficiency with or without anemia is frequent in IBD patients^[87], regular controls at 12 wk intervals are advisable so that treatment can be restarted promptly if needed.

Erythropoietin supplementation

Several studies have shown that recombinant human erythropoietin may be effective for treating ACD in IBD patients^[88-95]. In the anemia treatment algorithm, intravenous iron therapy should be considered as a first-line therapy in patients with severe anemia whereas erythropoietin treatment should be considered only in patients unresponsive to intravenous treatment, with low EPO levels, or who are unresponsive to aggressive management of IBD^[29,53,96] since EPO can be used as an adjunct therapy to control the inflammation^[97]. Recently, a prospective study on CD patients showed that EPO combined with enteral nutrition can improve the Hb levels in CD patients with a treatment success rate of 63.16% in the EPO group compared to none of the patients in the non-EPO group^[98]. When a decision has been made to administer EPO therapy, it should always be combined with intravenous iron supplementation to meet the increased demand caused by the “functional

iron deficiency” typical in IBD patients^[99].

Other treatments

Intramuscular vitamin B₁₂ continues to be the gold standard therapy for vitamin B₁₂ deficiency, especially in symptomatic patients^[100]. A dose of 1000 µg/wk for 8 wk, then 1000 µg once monthly for maintenance lifelong is recommended^[101]. No therapeutic advantages have been demonstrated for either cyanocobalamin or hydroxycobalamin in terms of serum levels during maintenance therapy^[102]. Effectiveness data for sublingual^[103,104] and intranasal^[105] routes for vitamin B₁₂ administration have revealed as promising non-invasive alternatives.

Specific treatment of IBD has been shown to gradually increase Hb levels over time, which indicates that the presence of anemia is positively associated with disease activity and disease-associated gut lesions. Some data suggest that anti-TNF-α treatment improves the anemia in a sub-group of patients with CD. In fact, patients who responded to treatment showed improvements in their anemia within 2 wk of the first infusion of Infliximab, with a parallel improvement in their CD activity index and an increase in endogenous EPO levels over time^[12]. Infliximab seems to neutralize the inhibitory effects of TNF-α on EPO production, increasing the availability of iron for erythropoiesis and reducing anemia^[30]. The drug produces these effects through various mechanisms, including reduced cytokine-induced formation of ferritin and hepcidin, with improvement of intestinal iron absorption and iron release from macrophages *via* ferroportin-mediated iron export^[21,106,107]. Moreover, Infliximab improved the proliferation of cultured BFU-E, blocking the inhibitory effects of cytokines on erythroid progenitor cells^[26]. Finally, it induced mucosal healing, thereby reducing the production of pro-inflammatory cytokines and the amount of blood loss through mucosal ulcers. Other therapies with potential for treating IBD associated with anemia include treatment with anti-IL-6, which is the major inflammation-driven inducer of hepcidin, and other new therapies that neutralize hepcidin, modify EPO and/or erythropoietin receptor sensitivity, or affect cytokines to effectively stimulate erythropoiesis.

The multi-factorial origin of anemia in IBD implies that several leading mechanisms can be simultaneously identified in a single patient, including chronic intestinal blood loss, decreased absorption capabilities of the small bowel secondary to inflammation or resection, bacterial overgrowth, and an inability of many IBD patients to tolerate the side effects of oral ferrous sulfate, among others^[108]. Each of these causative factors usually requires a specific therapeutic approach. Disease inflammatory activity and iron deficiency should be the first aspects to be restored in every patient^[109] since they are the main causes of anemia and easily identified. Although more uncommon, vitamin B₁₂ or folate deficiency, hemolytic and drug-induced anemia should also be born in mind. Effective treatment is only possible if the con-

tributing factors in a particular patient are clearly defined and corrected^[110].

CONCLUSION

Anemia is a common multifactorial complication in IBD that increases disease morbidity. Awareness on the part of gastroenterologists needs to be increased to improve the specific diagnosis and management of anemia in these patients. New generation IV iron compounds are currently available to treat iron deficiency effectively in IBD patients. Further studies are needed to establish standardized treatments to reduce the development and recurrence of anemia as well as to improve the clinical course of IBD.

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Psychiatric comorbidity in the treatment of patients with inflammatory bowel disease

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Abstract

Ulcerative colitis and Crohn's disease, commonly known as inflammatory bowel disease (IBD), draw attention from specialists of various disorders, including gastroenterology, psychiatry, and radiology. The involvement of a cortical influence in the brain-gut axis as well as the interaction of the hypothalamic-pituitary-adrenal axis and the peripheral nervous system provide an initial explanation of the psychological symptoms associated with IBD. The involvement of structures the limbic system, such as the anterior cingulate cortex, the prefrontal cortex, and the amygdala, paves the way for the discovery of the mechanisms underlying depression depression, anxiety, alexithymia, personality traits, and other psychological impairments following the onset of IBD. Psychiatric therapy in IBD patients is almost as important as the gastroenterological approach and consists of pharmacological treatment and psychotherapy. Neither of the available psychiatric treatment methods is considered the golden standard because both methods have side effects, and psychotropic medication can provoke the worsening of

IBD symptoms. Thus, both approaches must be applied with awareness of the possibility of side effects. We suggest that psychiatrists and gastroenterologists work together to reach a consensus on IBD therapy to ensure success and to reduce side effects and relapse to the lowest possible rates.

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Key words: Inflammatory bowel disease; Psychiatry; Treatment; Personality traits; Depression; Anxiety

Core tip: The involvement of a dysfunction of brain-gut interactions in the pathogenesis of inflammatory bowel disease (IBD) is represented by a dysfunction of the autonomic nervous system, an abnormal hypothalamic-pituitary-adrenal axis and cholinergic anti-inflammatory pathway, a deleterious effect of stress and depression, an abnormal coupling of the prefrontal cortex-amygdaloid complex, and an abnormal relation between the microbiota and the brain as pro-inflammatory factors. New investigations have provided a critical link between forebrain changes and abdominal pain independent of active disease and drug treatment, providing a potential basis for an explanation of the psychological symptoms and brain influence in the pathogenesis of IBD.

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ANATOMICAL BASIS FOR THE PSYCHIATRIC CHANGES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) results from an inap-

propriate inflammatory response to intestinal microbes in a genetically susceptible host. In recent reports, authors^[1,2] have discussed the involvement of a dysfunction of brain-gut interactions in the pathogenesis of IBD as represented by a dysfunction of the autonomic nervous system, an abnormal hypothalamic - pituitary - adrenal axis and cholinergic anti-inflammatory pathway, a deleterious effect of stress and depression as well as an abnormal coupling of the prefrontal cortex-amygdaloid complex and an abnormal relation between the microbiota and the brain as pro-inflammatory factors. The New investigations have provided a critical link between fore-brain changes and abdominal pain independent of active disease and drug treatment because all patients examined in the morphometric evaluation were in remission and suffered from ongoing abdominal pain. Investigators observed decreased gray matter volumes in the dorsolateral prefrontal cortex and the anterior midcingulate cortex (aMCC), and the disease duration was negatively correlated with volumes in the subgenual anterior cingulate (sACC), the posterior MCC (pMCC), the ventral posterior cingulate (vPCC), and the parahippocampal cortices. The aMCC has a role in feedback-mediated decision making, and specific cognitive tasks that differentiate the aMCC and pMCC can be used to evaluate defects in Crohn's disease (CD). The sACC is an important area because it has impaired functions in major depression. Because depressive symptoms are a feature in a subset of patients with active inflammatory diseases, including IBD, treatment targeting this subregion should prove efficacious. Finally, the vPCC has a role in ongoing self-monitoring of the personal relevance of sensory stimuli, including visceral signals *via* the sACC. This pathway may be interrupted by vPCC atrophy in CD. Cingulate atrophy in CD requires the targeting of chronic pain and psychiatric symptom therapies *via* neuronal circles involved in the cingulum. These therapies include psychotherapy, guided imagery and relaxation training, analgesic dosages of morphine or antidepressants, and hypnosis. Thus, a new generation of novel treatments may emerge from drug and non-traditional therapies for CD in this formative area of research^[3,4]. Nevertheless, a certain level of caution should remain: the same areas have been found to be susceptible to changes in temporal epilepsy^[5], and it remains unclear whether the volume alterations in these areas are specific to IBD or if they overlap with other diseases.

The white matter is not spared from damage in IBD patients. The number of such lesions is significantly higher in IBD patients compared to controls (12.75 ± 19.78 *vs* 3.20 ± 2.90 , $P < 0.05$). However, there are no significant differences between UC and Crohn's disease patients with regard to magnetic resonance imaging (MRI) findings. In addition, the incidence of white matter lesions and other brain parenchymal lesions, sinusitis, and otitis-mastoiditis does not differ significantly with disease activity ($P > 0.05$ for all)^[6].

Scheid *et al*^[7] (2007) proposed the following three possible mechanisms for peripheral and central nervous system involvement in ulcerative colitis (UC): cerebrovas-

cular conditions due to thromboembolic events, systemic and cerebral vasculitis, and neuropathy and cerebral demyelination due to immune-related mechanisms. In contrast, white matter lesion is a frequent finding in patients with IBD on MRI, and the development of these lesions has been attributed to ischemic mechanisms (atherosclerotic or vasculitic) or demyelination^[8-10]. Thus, early identification of these lesions may be clinically helpful as an early indication of neurological involvement because they may represent another extra intestinal manifestation of the disease^[10].

Studies performed by functional magnetic resonance imaging for both, patients and control subjects suffering from irritable bowel syndrome, which is also a psychosomatic disease, and control subjects, rectal distention stimulation increased the activity of the anterior cingulate cortex (number of positive answers to the stimulation/total number of patients: 35/37), the insular cortex (37/37), the prefrontal cortex (37/37), and the thalamus (35/37) in most cases. In patients with inflammatory bowel syndrome (IBS), the average percentage area of regions of interest increased in parallel with rectal distention volumes in the insular cortex, the prefrontal cortex, and the thalamic region. However, only the prefrontal cortex was statistically significant ($P < 0.05$). In controls, this tendency to increase only occurred in the anterior cingulate cortex. At 120 mL rectal distention, the average percentage area of regions of interest (ROI) and the average percentage change in MR signal intensity of ROIs in the insular cortex, the prefrontal cortex, and the thalamic region were significantly greater in patients with IBS than in control subjects^[11,12].

PSYCHOLOGICAL SYMPTOMS IN IBD

There is consistent evidence that psychological factors play a role in the pathophysiology and the course of IBD and in how patients cope with IBD^[12]. One prospective study in a population-based cohort of individuals with IBD ($n = 552$) evaluated whether the presence of a stressful event and the perception of stress as well as other factors (*i.e.*, nonsteroidal anti-inflammatory drugs, antibiotics, or infections) believed to contribute to triggering flares of IBD were, in fact, associated with symptomatic flares^[13]. Subjects completed surveys on health issues every 3 mo for 1 year. In any 3-mo period, approximately 50% of subjects experienced some type of stress, and the majority of subjects reported the stresses were everyday life stresses. Family stress was the most commonly reported stress, followed by work or school and financial stress. Subjects were grouped by disease activity over time. Significantly more individuals in the persistently inactive disease group indicated they experienced no stressful events compared with individuals in the persistently active disease group. In terms of the association between variables experienced in one 3-mo period and a symptomatic flare in the next 3-mo period, only psychological factors, including the occurrence of a major life event, high perceived stress, and high negative mood during a previous 3-mo period, were significantly

associated with the subsequent occurrence of a flare. This study complements the growing evidence from experimental as well as clinical studies that stress exposure, including stressful events and perceived stress (the individual's view of his or her own level of demand relative to resources), may contribute to relapse risk in IBD^[14-18]. In fact, using multivariate logistic regression analyses of these variables, only high perceived stress (adjusted OR = 2.40; 95%CI: 1.35-4.26) was associated with an increased risk of flare. This finding speaks to the bidirectional relationship between stress and symptomatic disease. Being symptomatic may exacerbate or even incite stress, whereas being stressed may trigger symptomatic disease. Recent reports have shown that brain derived neurotrophic factor (BDNF) levels are highly dynamic in response to stress. BDNF levels not only vary across brain regions but also fluctuate rapidly, both immediately after a stressor and over the course of a chronic stress paradigm. However, BDNF alone is not sufficient to effect many of the changes observed after stress. Glucocorticoids and other molecules have been shown to act in conjunction with BDNF to facilitate both the morphological and molecular changes that occur, particularly changes in spine density and gene expression^[19].

Although discrete personality traits have been studied in IBD patients, no specific personality type matches this disease. It is recommended that future research consider the discrete personality traits observed in these patients and integrate them in such a way that the traits will be addressed to include new personality types, such as types C and D^[20,21], which are well matched with the unregulated immune and hormonal systems that are characteristics of IBD. Type C individuals are introverted, perfectionistic, sensitive, and thoughtful. Individuals with a type D personality have the tendency to experience increased negative emotions across time and situations and tend to not share these emotions with others because of a fear of rejection or disapproval. It is believed that depression and anxiety are dominant in patients with IBD^[22]. These patients also have a higher prevalence of anxiety and depressive disorders than the general population but a lower prevalence of these disorders than patients with a functional bowel disorder. The prevalence (21%-35%) is similar to that found in other patients with chronic physical illness. Depressive disorder appears to be more common in older patients and individuals with a previous history of a psychiatric disorder^[23,24]. Patients suffering from IBD take more medications than the healthy population: the use of antidepressants (OR = 1.44, 95%CI: 1.28-1.61), anxiolytics (OR = 1.52, 95%CI: 1.31-1.78), oral bisphosphonates (OR = 6.08, 95%CI: 4.56-8.11), cardiovascular medications (OR = 1.38, 95%CI: 1.24-1.54), antibiotics (OR = 4.01, 95%CI: 3.57-4.51), proton pump inhibitors (OR = 3.90, 95%CI: 3.48-4.36), and nonsteroidal anti-inflammatory analgesics (OR = 1.17, 95%CI: 1.07-1.28) is significantly more common in IBD patients than in controls. Individuals who use antidepressants, anxiolytics, or analgesics have significantly impaired health-rated quality of life (HRQOL) ($P < 0.001$)^[25].

Both depression and anxiety precede ulcerative colitis significantly more often than would be predicted from the control population's experience^[24]. The association is strongest when the two psychiatric disorders and ulcerative colitis are diagnosed in the same year, although the association between depression and ulcerative colitis is also significant when depression precedes ulcerative colitis by five or more years. Neither depression nor anxiety precedes Crohn's disease more often than expected by chance, although the study involved fewer cases with Crohn's disease than ulcerative colitis. Two prospective clinical studies of patients with IBD appear to produce conflicting results. During a 6-mo follow-up period, one study found a strong association between the change in disease activity and anxiety level and a weaker association with depressive symptoms. Changes in disease activity seemed to lead to changes in anxiety and depression. Beck Depression Inventory scores at baseline predicted the number and timing of relapses during an 18-mo follow-up period^[26,27].

Nevertheless, the origin of depression and anxiety in patients with IBD remains at least insufficiently explained. In a review of psychotherapeutic approaches to IBD, Prasko *et al*^[28] (2010) emphasized that higher scores of neuroticism, depression, inhibition, and emotional instability are typical for many patients with chronic diseases and nonspecific for chronic gastroenterological disorders. More directly, anxiety and depression are consequences of IBD symptoms, such as frequent stools with evidence of blood, pain in the abdominal region, and bloating.

Because anxiety and mood disorders are the most common mental health concerns in the community, these disorders are usually the focus of screening efforts. The typical symptoms of anxiety disorders include high levels of physiological arousal, excessive worries about the future, avoidance of feared situations (including, in some cases, medical appointments and procedures)^[29,30], and difficulty coping with unfamiliar situations. Depression typically presents with a constellation of affective, cognitive, and somatic symptoms, including a sad or depressed mood; a loss of interest in normal activities; feelings of guilt, worthlessness, or hopelessness; difficulties with concentration; reduced energy; changes in appetite and sleep; and withdrawal from usual activities. When these clusters of symptoms persist beyond a few weeks and begin to significantly interfere with daily functioning, they are typically considered to reach a clinical threshold^[31]. The incidence of IBD in adolescents has been reported to be an increasing trend, especially in the Northern parts of Europe (Finland, Scotland)^[32-34]. According to parent reports, adolescents with IBD have more emotional, social, and thought problems and lower competence than their healthy peers^[35]. The disease disturbs adolescents' quality of life^[25,36,37], may have negative consequences for education and school functioning^[38,39], and may be a cause of difficulties in employment, such as finding or maintaining a desired job^[40-42]. Furthermore, adolescents with severe IBD have disturbed sleep and are overtired

more often than their healthy peers^[43].

Some studies have shown alexithymia to be another common personality characteristic in IBD patients. Patients with alexithymia have difficulties in recognizing and verbalizing emotions, and their ability to regulate emotions and express them to others is usually reduced^[44,45]. Numerous studies^[46-50] have shown that IBD patients have higher scores for alexithymia than controls. In a study conducted by Jones *et al.*^[46] (2006), the scores of 74 IBS patients, 55 healthy control subjects, and 48 IBD patients were compared on the Toronto Alexithymia Scale. The results showed that IBS and IBD patients had higher scores on measures of alexithymia than the controls, but they did not differ from one another. In an epidemiological study, Porcelli *et al.*^[50] (1999) compared 121 functional gastrointestinal disorder patients, 116 IBD patients, and a group of 112 healthy subjects using the Toronto Alexithymia Scale. Their results showed that the FGID group was significantly more alexithymic than the IBD group, and the scores of the two gastrointestinal groups were higher than the normal healthy group. Even after controlling for the influence of education, gender, anxiety, depression, and gastrointestinal symptoms, these differences remained significant. Moreno-Jiménez *et al.*^[47] (2007) did not use a control group. In their sample comprising 60 UC and 60 CD patients, they attempted to address the question of which personality factors may predict HRQOL in IBD patients. They showed that difficulty in describing one's feelings was significant for predicting two dimensions of HRQOL, systemic symptoms and social functioning. Difficulty in describing one's feelings negatively predicted systemic symptoms and social functioning. Patients experiencing more difficulty in describing their feelings reported lower HRQOL. Although alexithymia may not be specific to IBD, it may lead patients to communicate their psychological distress through somatic and behavioral symptoms rather than verbal communication. This may occur particularly when patients have limited perceived social support or personality traits such as introversion. Regardless of whether alexithymia is specific to IBD, it has been reported that affected patients have greater difficulty in describing their feelings to others, poorer disease outcome, lower psychological functioning, and worse HRQOL^[12,47,51].

Adolescents with IBD have mild cognitive problems compared to the same population with juvenile idiopathic arthritis, particularly in the acute phase. Adolescent patients with IBD produced more perseverative errors than patients with non-acute juvenile idiopathic arthritis. Perseveration in the California Verbal Learning Test may be related to a momentary loss of alertness in the tiresome and long verbal memory test. However, no other differences in cognitive functioning between the study groups were detected. These findings indicate that adolescents with active IBD may have some mild problems in verbal memory but no major cognitive deficits. Prior studies in adults with IBD found deficits, particularly in verbal functioning, suggesting that in the clinical evaluation of young patients with IBD, it may be relevant to pay atten-

tion to even minor cognitive problems that may be aggravated during the growth process^[43,52,53].

PSYCHIATRIC THERAPY IN IBD

Psychiatric treatment of patients with IBD involves two types of approaches: (1) psychotropic medication; (2) psychotherapy; and (3) psychotropic medication.

Anxiety and depression, the most common psychiatric symptoms in IBD patients, are highly treatable conditions. The interventions that are the most widely used and have been evaluated the most extensively for anxiety and depressive disorders are specific pharmacological agents [particularly selective serotonin reuptake inhibitors (SSRIs), such as citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline, and the serotonin norepinephrine reuptake inhibitor (SNRI) venlafaxine] and specific psychological treatments (particularly cognitive behavioral therapies). The SSRI and SNRI medications are second-generation antidepressants that have been well established in the literature as being similarly effective for anxiety and depression^[54].

In humans, it has been observed that although antidepressants improve both the mental and somatic status of IBD patients, the low quality of available research provides significant barriers to making a definitive statement on their efficacy or lack thereof^[55]. Animal models, however, have found a positive impact of desipramine and fluoxetine on inflammation in models of IBD. When doctors' perspectives on antidepressants in IBD were examined, it was reported that gastroenterologists commonly treat IBD patients with antidepressants for pain, anxiety and/or depression, and insomnia. Gastroenterologists reported that antidepressants were successful in reducing pain, gut irritability, and urgency of defecation. In the most recent retrospective case-note audit of 287 patients, 83 (28.9%) patients had used an antidepressant at some time in their life^[56-59]. Nonetheless, the design of the study does not allow a firm statement to be made about whether antidepressants improved the course of IBD. The recent study in this area conducted by Goodhand *et al.*^[60] (2012), which examined the disease course one year before and one year after the commencement of antidepressants, showed that patients reported fewer relapses and steroid treatment in the year after starting an antidepressant than in the year before. This effect was not observed in the control group. Although this report of decreased symptoms may simply reflect the report of fewer functional gastrointestinal symptoms when patients are in better psychological health^[61,62], it may also indicate an inflammation-specific benefit from antidepressants. Thus, it is clear that randomized controlled trials are justified and needed to provide a definitive answer regarding the efficacy of antidepressants in IBD.

Amitriptyline is an antidepressant drug that is widely used for the treatment of IBD and gastrointestinal disorders^[55,63]. It is effective for treating psychological and somatic symptoms in patients suffering from IBD^[55]. Other studies have shown the anti-inflammatory effects

of antidepressants by different mechanisms^[64,65]. Amitriptyline also acts on $\alpha 1$ -adrenoceptors to produce anti-inflammatory effects^[64]. Due to its effects on the inhibitory cytokine interleukin-10, amitriptyline has been reported to suppress neuroinflammation^[66]. Furthermore, antidepressants have anti-inflammatory effects by considerably decreasing the production of nitric oxide (NO) and tumor necrosis factor- α (TNF α) in microglia and astrocyte cultures at mRNA levels^[65]. They can inhibit the degradation of I κ B, the nuclear translocation of the p65 subunit of NF- κ B. Therefore, NF- κ B cannot translocate into the nucleus to bind with DNA to promote the expression of gene regions^[66]. Antidepressants can also inhibit the phosphorylation of p38 mitogen-activated protein kinase in lipopolysaccharide-stimulated microglia cells^[65]. This phosphorylation can induce the associated inflammatory gene expression to produce the proinflammatory cytokines and NO, which may be attenuated or inhibited by antidepressants^[66]. NO can also induce ROS; therefore, it can increase intestinal damage and, with cytokine production, prolong the development of IBD. Based on these studies, the NF- κ B pathway has been considered to play an important role in the inflammatory process. Therefore, investigators have hypothesized that the antidepressant-like effects of amitriptyline, *via* the modulation of this pathway, may be more effective for treating and suppressing the development of IBD through its anti-inflammatory actions^[67].

Some authors have argued that antidepressants may, in fact, cause tolerance and present problems when tapering off medications^[68,69]. Significant numbers of patients no longer need an antidepressant for their mental health problems, yet they suffer unbearable withdrawal effects when discontinuing the medication and thus remain on the treatment^[58]. Patients participating in a study by Mikocka-Walus *et al.*^[58] (2012) reported antidepressants to be a medication worth recommending to fellow IBD sufferers as long as the decision for their use was taken into consideration. Although studies exploring attitudes toward antidepressant use in larger samples or in samples recruited in primary care (and thus with possibly better-controlled IBD) are not available, studies conducted in the general population in primary care have shown a less receptive attitude toward antidepressants. For example, a survey of 1054 primary care users showed that over 20% of individuals did not disclose depressive symptoms to their doctors due to fear that antidepressants would be prescribed^[70]. Other studies have reported non-adherence to treatment with antidepressants due to patient beliefs or misconceptions about this type of medication^[71,72]. In light of these findings, the positive attitudes toward antidepressants identified in previous studies should be interpreted with caution and confirmed by larger quantitative studies with more representative IBD samples.

A systematic review of SSRIs indicated that although the medications were similar in efficacy, there were meaningful differences in their side effect profiles. These differences may guide decisions concerning the best choice for a particular patient^[73]. Nevertheless, studies report

serious adverse effects of selective serotonin reuptake drugs. Gastrointestinal side effects can be of particular concern to the IBD patient and have been reported for many antidepressant medications. These side effects are generally dose related and tend to decrease over the first weeks of treatment^[74]. Nausea and vomiting are more frequent with the one SNRI evaluated (venlafaxine) compared to the SSRIs as a group (fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram; 34% *vs* 22%). Diarrhea is reported more often with sertraline than with the other SSRIs. Other side effects that have been cited as problematic when patients decide to discontinue antidepressant medication early in the course of treatment include drowsiness/fatigue (10%), anxiety (6%), headache (6%), insomnia (2.7%), and dizziness (2.7%)^[75]. Weight gain has been found to be a more significant problem with paroxetine and mirtazapine^[74], but it remains a concern with all SSRI medications^[76]. In some cases, there may be weight loss early in treatment and weight gain later^[74]. Decreased sexual functioning is a relatively common dose-related side effect of antidepressant medications and may be a concern for IBD patients, given the disease-related difficulties with intimacy and sexual functioning^[77]. It has been found that 60% to 70% of patients report reduced sexual functioning on SSRIs or SNRIs that does not improve with longer periods on the medication. Bupropion has the lowest rates of sexual dysfunction relative to other antidepressants^[76], and it is usually recommended as a substitute for SSRIs in case of side effects. Kast *et al.*^[78] (2001) reported two patients who achieved long-lasting remission of Crohn's disease while using bupropion. These investigators hypothesized that this outcome may have resulted from decreased TNF α , which is known to play a vital role in Crohn's disease. Phenelzine and bupropion increase intracellular cyclic adenosine mono-phosphate^[79], which, in turn, decreases TNF α . Because phenelzine may cause a hypertensive crisis, bupropion is suggested to be a safer therapeutic option than phenelzine. Interestingly, phenelzine and other monoamine oxidase inhibitors have been noted to induce remission of rheumatoid arthritis, a disease in which, as in Crohn's disease, TNF α has a central role^[80]. Kast^[81] (2003) compared the use of bupropion and mirtazapine in patients with Crohn's disease. He speculated that both of these antidepressants have the potential to affect inflammatory responses: bupropion by lowering TNF α and mirtazapine by increasing its level. Therefore, according to the hypothesis of Kast^[81] (2003), there are theoretical reasons for recommending bupropion and cautioning against mirtazapine when treating depression in patients with Crohn's disease. Although Kast's explanations appear logical and are supported by other investigators^[82], their practical effectiveness needs to be experimentally confirmed in appropriate clinical studies^[55].

With regard to the risk of severe side effects, recent reports have raised the possibility of a greater risk of upper gastrointestinal (GI) bleeds with the use of certain antidepressants^[83-85]. Large-scale studies have found a moderately increased risk of GI bleeds with SSRIs as well as with

the SNRI venlafaxine^[86-88]. The use of acid-suppressing agents mitigated the higher risk, whereas the use of non-steroidal anti-inflammatory drugs (NSAIDs) increased the risk^[86]. The absolute risk of taking SSRIs was low, however; 2000 patients per year would need to be treated with SSRIs for one case of upper GI tract bleeding to be attributed to such drugs. The risk is higher when SSRIs and NSAIDs are taken together, with one patient in 250 experiencing a GI bleed that could be attributed to that combination^[86].

PSYCHOTHERAPEUTIC APPROACH

The first study regarding the effectiveness of psychotherapy for ulcerative colitis was conducted half a century ago^[89], but it was methodologically problematic. The effect of psychodynamic psychotherapy on patients with Crohn's disease was investigated in a randomized, multicenter study^[90]. The psychotherapeutic intervention consisted of psychodynamic psychotherapy (26 sessions) and autogenic training (17 sessions). After 2 years, relapse was not experienced by 23% of the control group and 30% of the therapy group. Twenty-nine percent of the control group and 17% of the therapy group had to undergo surgery. The therapy group had better somatic data than the control group, but this was not significant. Nevertheless, a meta-analysis was necessary for this review article given that the authors analyzed a respectable number of investigations addressing the subject of interest, such as the use of psychotherapy in IBD patients^[91,92]. Twenty-one studies were included in the analysis. Four studies did not report results in detail and could not be included in any of the pooled analyses. These 4 studies included a controlled trial on hypnosis in ulcerative colitis^[93], a trial on the effects of a multicomponent behavioral therapy package in 22 patients with IBD^[94], and a trial examining the effects of support meetings^[95].

Given its chronic nature and frequently reported poor quality of life for many patients, IBD is often associated with anxiety and depression. In addition to medical treatment, psychological intervention may be a crucial component of the treatment of IBD patients^[96,97]. Prior research has found that the disease course is gender dependent and that females may have a higher risk of disease activity relapse than males^[22,98]. Therefore, females may have more psychological symptoms. Furthermore, females more frequently become ill from Crohn's disease. Second, patients are stratified by treatment center. A distinction must be made between an academic setting and a peripheral setting. We expect that an academic hospital would treat more severe cases of IBD than a peripheral setting. Finally, the disease type is also used as a stratification factor. Previous research has found that CD patients undergo more surgical interventions and experience more disease exacerbations than ulcerative colitis patients^[99]. This finding indicates that CD is a more complex disorder than UC. In addition, CD patients report poorer quality of life and more anxiety symptoms than UC patients^[99,100]. Therefore, it is important that UC and CD patients are distributed evenly in the experi-

mental group and the waiting list control group^[101]. Of all psychotherapeutic interventions, cognitive behavioral therapy appears to be the most effective^[102]. This therapy posits that an individual's biased information processing leads to restrictive thoughts, feelings, and behaviors that can culminate in anxiety and depressive symptoms and, eventually, in psychiatric disorders. Cognitive behavioral therapy offers a well-developed intervention protocol that has been found to enhance quality of life and to decrease psychological distress. Its positive effect has been emphasized in individuals with other chronic somatic illnesses, such as chronic obstructive pulmonary disease, diabetes, and cancer^[103,104].

Cognitive behavior therapy was evaluated in an open trial of adolescents with major depression^[105,106] and a randomized controlled trial (RCT) of adolescents with subsyndromal depression^[107]. In all studies, treatment significantly reduced depression and improved global functioning. For individuals with a comorbid anxiety disorder, there was also a significant reduction in anxiety. The open trial did not find any change in illness severity posttreatment. The RCT reported a decrease in the number of individuals with moderate to severe disease posttreatment (29% pre-treatment *vs* 15% post-treatment), but the decrease was not significant. In an RCT of adults with IBD, a Spanish group reported clinically significant reductions in anxiety and depression following a structured cognitive-behavior therapy program that included components such as relaxation training, distraction, and cognitive restructuring^[108].

Reviews considering the overall effectiveness of psychological therapies for IBD patients not selected for anxiety or depressive disorders have reached a more modest conclusion: there may be some clinical benefit related to psychological functioning, with little support at this point for a significant direct impact on disease parameters^[109-111]. These studies incorporated a broad range of treatments (*e.g.*, psychodynamic therapy, supportive therapy, and cognitive behavioral therapies), some of which are not as well supported empirically. Further, the studies often involved unselected IBD patients or patients in remission with little elevated distress^[112,113], resulting in the potential for floor effects. Thus, unsurprisingly, psychological treatment is not indicated for all patients with IBD^[114]. The results of well-conducted studies imply that validated treatments should be targeted to high-risk subgroups, such as individuals with comorbid psychiatric conditions or elevated stress^[110,111]. Certainly, IBD patients may be quite susceptible to psychological treatment. Among individuals reporting high distress, there is a strong level of interest in receiving support for these concerns^[115]. A structured measure of desire for psychological care comparing patients with IBD and rheumatoid arthritis found that 2 to 3 times the number of IBD patients (31%) expressed an interest in receiving assistance compared to individuals with rheumatoid arthritis (13%)^[116]. Other indicators of receptivity included positive evaluations of treatment^[113] and low dropout rates despite the expectation of active participation^[104,106].

The appropriate choice of medication depends on many factors that are best tailored to the individual patient. Different galenic preparations are released at different sites and may have local activity [such as mesalazine (5-ASA) preparations, budesonide, or types of enemas]. The choice is influenced by the balance between drug potency and side effects, previous response to treatment (especially when considering treatment for a relapse or treatment for corticosteroid-dependent or corticosteroid-refractory disease), and the presence of extraintestinal manifestations (indicating the need for systemic therapy) or complications. Despite general agreement that treatment decisions for active Crohn's disease should be based on the site as well as the activity and behavior of the disease, the sample size is too small for statistically valid conclusions to be drawn from therapeutic trials when patients are stratified according to the site of disease^[117].

For mildly active IBD, budesonide 9 mg/d is favored because it is superior to both placebo (OR = 2.85, 95%CI: 1.67-4.87)^[118,119] and 5-ASA 4 g/d (OR = 2.8, 95%CI: 1.50-5.20)^[120], and it achieves remission in 51%-60% of individuals over 8-10 wk^[119,121-123]. Budesonide is preferred to prednisolone for mildly active CD because it is associated with fewer side effects, although a Cochrane systematic review has shown budesonide to be somewhat less effective than prednisolone (pooled OR for the five trials 0.69, 95%CI: 0.51-0.95)^[119]. For corticosteroid-related adverse effects, budesonide showed no difference from the placebo (OR = 0.98, 95%CI: 0.58-1.67)^[118,119] but had fewer side effects than prednisone (pooled OR = 0.38, 95%CI: 0.28-0.53).

When IBD is estimated as moderately active, budesonide or prednisolone are appropriate. Prednisolone is associated with a good clinical response (92% remission within seven weeks at a high dose of 1 mg/kg), but it commonly causes more side effects than budesonide^[117,124,125]. The dose of prednisolone is adjusted to the therapeutic response over a period of weeks. More rapid reduction is associated with early relapse. The consensus does not favor sole nutritional therapy, antibiotics (unless septic complications are suspected), infliximab (IFX) (until more data are available), or surgery for moderately active ileal CD as the first line therapy^[122].

Prednisolone or intravenous hydrocortisone is appropriate for the initial treatment of severe ileal CD. Azathioprine (AZA) (or mercaptopurine) should be added for individuals who have relapsed because it has a corticosteroid-sparing effect (NNT 3) and is effective at maintaining remission^[126,127]. Methotrexate should be considered an appropriate alternative if thiopurines cannot be tolerated, but it has specific contraindications, such as pregnancy. IFX is best reserved for patients who do not respond to initial therapy and for whom surgery is considered inappropriate. This does not mean that surgery takes precedence over IFX. IFX has emerged as a conservative option for cases with severe inflammatory activity, and it is in these cases that primary surgery will often be inappropriate. Surgical options should, however, be considered and discussed with the patient as part of

an overall management strategy. The stage at which IFX is introduced may change if it can be established whether early therapy changes the pattern of disease. The threshold for surgery for localized ileocecal disease is lower than for disease elsewhere, and some experts advocate surgery over IFX for disease in this location. Other experts advocate resection if medical therapy is not effective within two to six weeks. It may sometimes be difficult to distinguish between active disease and a septic complication, but antibiotics should be reserved for patients with a fever or focal tenderness or in whom imaging has indicated an abscess. Adding ciprofloxacin and metronidazole to budesonide has been shown to have no advantage over budesonide alone in active CD^[127,128].

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Regular infusions of IFX 5 or 10 mg/kg every eight weeks are effective at maintaining an IFX-induced response in nonfistulating CD (EL1b). Patients in a scheduled treatment strategy with regular infusions of IFX seem to fare better in many (but not all) clinical end points compared with patients in an episodic (on-demand) strategy^[129,130].

CONCLUSION

The psychopathological impact in IBD patients is evident, and the most common symptoms of depression and anxiety may affect the success of the desired gastroenterological therapy. Personality traits, cognitive impairment, and sleep deprivation are the tip of the iceberg of psychological problems in patients with IBD. The worsening of psychological problems is often followed with the same trend in GI symptoms of IBD and vice versa; that is, relapse of IBD symptomatology, such as blood in the stool, bloating, and pain, may increase psychological problems. This "vicious cycle" could be broken by involving a trained psychiatrist in the IBD treatment team. Nevertheless, some measures of precaution should be taken, such as avoiding bias in the group selection for both psychotropic medication and psychotherapy and an awareness of the possible side effects of antidepressant therapy, especially SSRIs, such as more frequent liquid stools, addiction, or sexual dysfunction problems. Finally, we suggest that psychiatrists and gastroenterologists work together to determine the final consensus of the IBD therapy to ensure success and to reduce side effects and relapse to the lowest possible rates.

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Colon-specific prodrugs of 4-aminosalicylic acid for inflammatory bowel disease

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Author contributions: Dhaneshwar SS has conceptualized, drafted and compiled this comprehensive review through extensive survey of the available literature and databases, adding a new perspective based on her expertise in this area.

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Abstract

Despite the advent of biological products, such as anti-tumor necrosis factor- α monoclonal antibodies (infliximab and adalimumab), for treatment of moderate to severe cases of inflammatory bowel disease (IBD), most patients depend upon aminosalicylates as the conventional treatment option. In recent years, the increased knowledge of complex pathophysiological processes underlying IBD has resulted in development of a number of newer pharmaceutical agents like low-molecular-weight heparin, omega-3 fatty acids, probiotics and innovative formulations such as high-dose, once-daily multi-matrix mesalamine, which are designed to minimize the inflammatory process through inhibition of different targets. Optimization of delivery of existing drugs to the colon using the prodrug approach is another attractive alternative that has been utilized and commercialized for 5-aminosalicylic acid (ASA) in the form of sulfasalazine, balsalazide, olsalazine and ipsalazine, but rarely for its positional isomer 4-ASA - a well-established antitubercular drug that is twice as potent as 5-ASA against IBD, and more spe-

cifically, ulcerative colitis. The present review focuses on the complete profile of 4-ASA and its advantages over 5-ASA and colon-targeting prodrugs reported so far for the management of IBD. The review also emphasizes the need for reappraisal of this promising but unexplored entity as a potential treatment option for IBD.

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Key words: 4-Aminosalicylic acid; 5-Aminosalicylic acid; Sulfasalazine; Colon-specific prodrug; Inflammatory bowel disease; Ulcerative colitis; 2,4,6-trinitrobenzene sulphonic acid; Experimental colitis

Core tip: Anti-inflammatory activity of antitubercular drug 4-aminosalicylic acid (ASA) was first described by Lover in 1984. Since then, numerous clinical trials were carried out to establish its efficacy in left-sided and active/quiescent ulcerative colitis. It is 50% more potent than 5-ASA against inflammation and does not produce 5-ASA-induced immunoallergic acute pancreatitis. 4-ASA is a stable and inexpensive alternative to 5-ASA in patients with acute pancreatitis. Despite all these positive findings, an extensive literature review surprisingly revealed few colon-targeting delivery systems for 4-ASA. The present review presents a complete profile of 4-ASA and its colon-specific prodrugs for inflammatory bowel disease.

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INTRODUCTION

Anti-inflammatory activity of antitubercular drug 4-ami-

nosalicyclic acid (ASA) was first described by Lover in 1984. Since then, numerous clinical trials were carried out to establish its efficacy in left-sided and active/quiescent ulcerative colitis. It is 50% more potent than 5-aminosalicylic acid (5-ASA) against inflammation and does not produce 5-ASA-induced immunoallergic acute pancreatitis. 4-ASA is a stable and inexpensive alternative to 5-ASA in patients with acute pancreatitis. Despite all these positive findings, an extensive literature review surprisingly revealed few colon-targeting delivery systems for 4-ASA. The present review presents a complete profile of 4-ASA and its colon-specific prodrugs for inflammatory bowel disease (IBD).

IBD

Ulcerative colitis (UC) and Crohn's disease (CD) are two distinct idiopathic, chronic and relapsing inflammatory disorders of the gastrointestinal tract (GIT) that are grouped under the term IBD. Dysregulation of immune responses and upregulation of proinflammatory mediators are the two hallmarks of IBD that make it recurrent and nearly incurable^[1]. UC is characterized by distinct episodes of active and inactive disease in 80%-90% of patients. Repetitive cycles of active and quiescent disease with varying clinical patterns are also observed in CD^[2].

UC is one of the most common chronic inflammatory forms of IBD and is characterized by diffused mucosal inflammation, mainly limited to the large intestine and rectum. UC is characterized by diarrhea, rectal bleeding and abdominal pain. Inflammation of the rectum is common and generally extends proximally in a continuous fashion^[3]. CD is a patchy transmural granulomatous inflammation that affects any part of the GIT and has a predilection for the terminal ileum and colon^[4].

Despite the differences between UC and CD, both forms of IBD cause similar symptoms. UC is characterized by mild symptoms like progressive loosening of the stools, abdominal cramping, and diarrhea. In the severe form of the disease, patients may also experience weight loss, fatigue, and loss of appetite that may result in nutrient deficiencies, mucus in the stools, severe rectal bleeding, fever, and anemia. In CD, abdominal pain, diarrhea and weight loss are often the earliest signs; constitutional symptoms include malaise, lethargy, anorexia, nausea, vomiting and low-grade fever. CD can be complicated by the development of intestinal obstruction, fistulae and perianal disease. Fistulae develop in about one-third of patients. Perianal disease is a frequent complication of colonic and ileocolonic disease and is characterized by fissures, fistulae and abscesses^[5,6].

The pathogenesis of IBD is obscure and is considered to involve multifactorial interactions amongst genetic, immunological and environmental triggers. The key factor differentiating individuals with IBD from normal ones is their inability to downregulate the uncontrolled and chronic inflammatory state. The pathological findings associated with IBD are: an increase in certain inflammatory mediators, signs of oxidative stress, a deranged colonic milieu, abnormal glycosaminoglycan content of the

mucosa, decreased oxidation of short chain fatty acids, increased intestinal permeability, increased sulfide production, and decreased methylation. Although no single factor has been identified as the initial trigger for IBD, the etiological factors have been elucidated^[7]. However, no guaranteed curative therapeutic regimen has been developed so far.

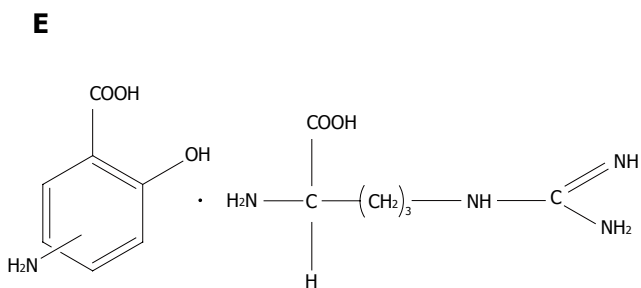
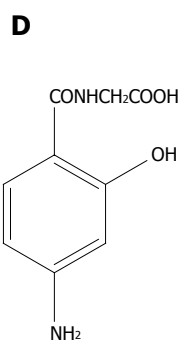
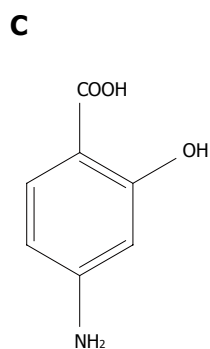
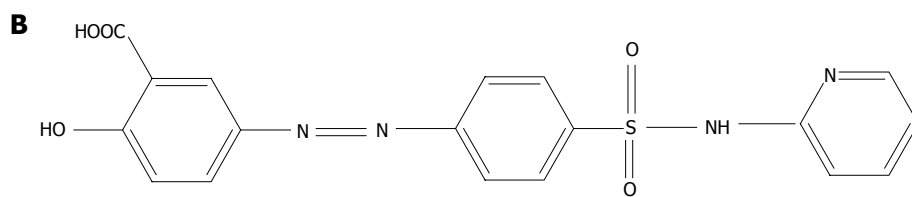
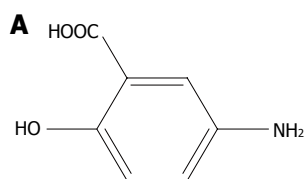
IBD THERAPY

Therapy for IBD has not seen major changes or breakthroughs during the past 4-5 decades and still revolves around the single nonsteroidal anti-inflammatory drug 5-ASA (Figure 1A) and its colon-targeting prodrug sulfasalazine (Figure 1B), followed by second-line treatment with steroids and immunomodulators, because reducing the extent and severity of colonic inflammation remains the primary focus of treatment. Antibiotics, probiotics, and nutritional supplements are used as supportive therapy. The use of anti-tumor necrosis factor monoclonal antibody (infliximab), recombinant anti-inflammatory cytokines, and related gene therapy are recent advances in the field^[8,9].

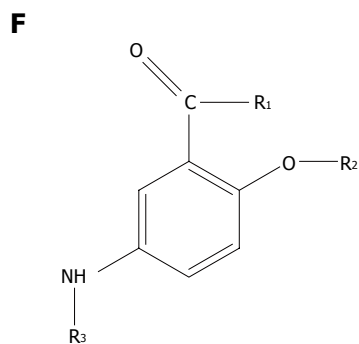
High and repeated doses of 5-ASA are necessary for maintenance and preventive therapy of IBD relapse. The majority of orally administered 5-ASA is readily and extensively absorbed from the stomach and small intestine, leaving a small amount that is transported to the colon. 5-ASA is absorbed from the upper GIT, causes many systemic side effects, and at the same time, results in poor bioavailability at the site of action, namely, the colon. Bypassing absorption in the upper GIT and targeting the delivery of 5-ASA to the colon by designing colon-specific prodrugs is the rational approach to improve the bioavailability at the site of action. The earliest reported colon-specific prodrug of 5-ASA is sulfasalazine, which is extensively prescribed for the treatment of UC. It is an azo conjugate of 5-ASA with sulfapyridine. However, its sulfapyridine part is used as a carrier and is completely absorbed through the colon and produces adverse effects such as hypersensitivity, impotency, blood dyscrasia, hepatitis, abnormal liver function tests and hepatic failure, agranulocytosis, leukopenia, thrombocytopenia, hemolytic anemia, and cyanosis^[10-12]. Although 5-ASA derivatives have an excellent safety profile, some undesirable effects may occur; the most prominent being immunoallergic acute pancreatitis^[13,14]. These side effects contraindicate the further use of all 5-ASA-containing drugs and pose a problem, because they limit treatment of acute phases of the disease to corticosteroids and relapse to immunosuppressants. 4-ASA has also been shown to be effective in IBD; in enemas or in oral formulations^[15-18].

4-ASA

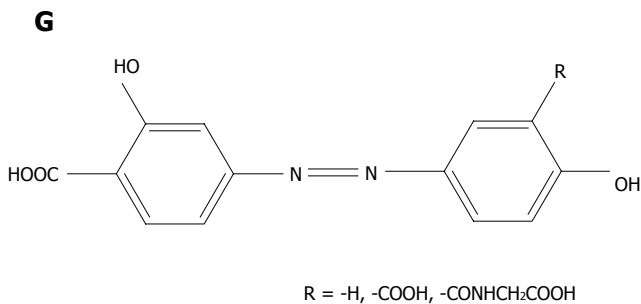
4-ASA (p-aminosalicylic acid, 4-amino-2-hydroxybenzoic acid) (Figure 1C), is a comparatively safe antitubercular agent that has been used for many years in resorbable high oral doses (ranging from 8 to 12 g/d) in multidrug-resistant tuberculosis. It has a bacteriostatic effect on



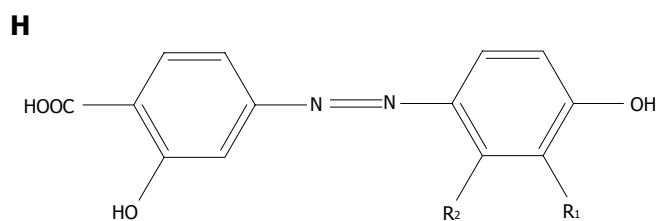
Wherein: -NH₂ is either at position 4 or 5



Where, R₁, R₂, R₃ = H₂S releasing moiety

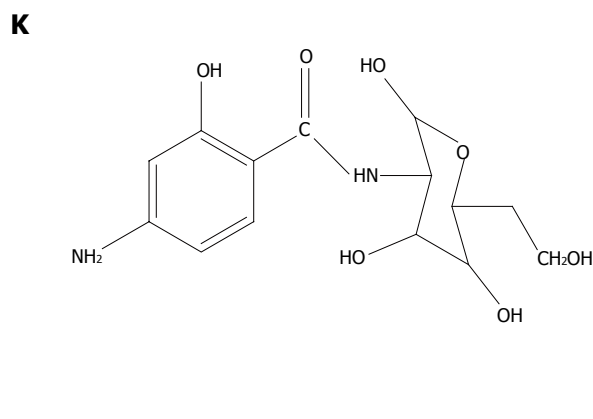
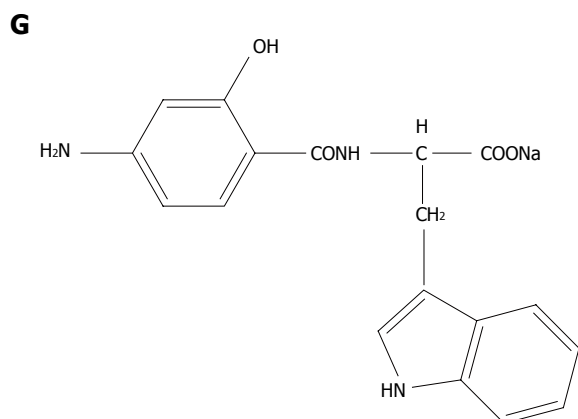
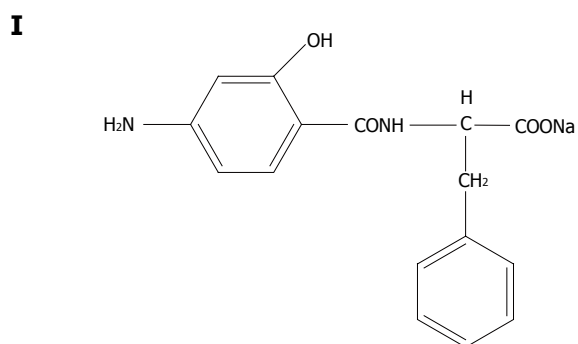


R = -H, -COOH, -CONHCH₂COOH



Where, R₁ = -H, R₂ = -CH₃; R₂ = -NO₂

R₂ = -H, R₁ = -CONH₂; R₁ = -Cl, R₁ = -CH₃; R₁ = -NO₂



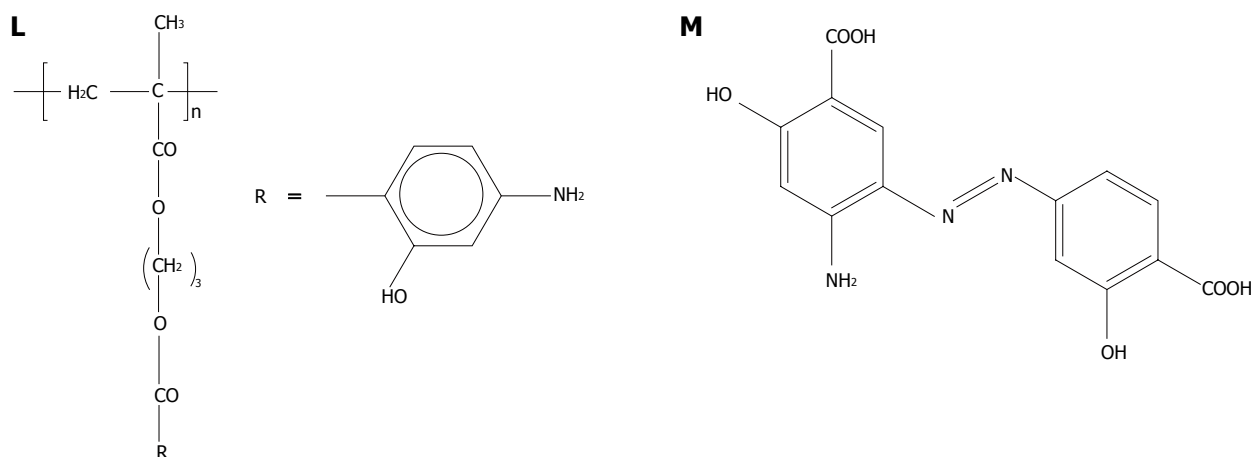


Figure 1 Chemical structures of aminosalicylates and their colon-targeting prodrugs. A: 5-ASA; B: Sulfasalazine; colon-targeting prodrug of 5-ASA with sulfa-pyridine; C: 4-ASA; D: Amide prodrug of 4-ASA with glycine; E: L-arginine salts of 4-ASA and 5-ASA; F: H₂S-releasing prodrugs of 5-ASA and 4-ASA; G: Prodrugs of 4-ASA with salicylic acid, hydroxybenzene and N-salicyloyl glycine; H: Prodrugs of 4-ASA with substituted phenols; I: amide conjugate of 4-ASA with D-phenylalanine; J: Amide conjugate of 4-ASA with L-tryptophan; K: Amide prodrug of 4-ASA with D-glucosamine; L: Polymeric prodrug of 4-ASA; M: Prodrug of 4-ASA with 4-ASA (4AAz).

Mycobacterium tuberculosis and inhibits resistance against streptomycin and isoniazid. Chemically, it is distinguished from its position isomer 5-ASA by the position of the NH₂ group, although they share the salicylic acid backbone. Therefore, their method of synthesis is also different. 4-ASA is prepared by heating 3-aminophenol with potassium bicarbonate or ammonium carbonate under pressure, while 5-ASA is prepared by zinc/hydrochloric acid-assisted reduction of m-nitrosalicylic acid^[19]. 5-ASA is unstable and is degraded rapidly into a dark purple quinone containing tar but does not decarboxylate in the presence of moisture. However, 4-ASA is readily decarboxylated in the presence of moisture, but unlike 5-ASA, is slowly degraded into brown to purple material^[20,21]. In view of the well-recognized chemical distinction between 4-ASA and 5-ASA, it is surprising to discover that not only does 4-ASA have anti-inflammatory activity, but it is also about 50% more potent than 5-ASA.

Lover reported in animal models that 4-ASA may have anti-inflammatory properties comparable with those of 5-ASA^[22]. Numerous clinical studies have shown that 4-ASA is highly effective in the topical treatment of active ulcerative proctitis or active left-sided UC^[23]. Moreover, slow release tablets of 4-ASA are effective in active UC^[17]. 4-ASA has been suggested as an effective treatment for both active and quiescent UC. 5-ASA is well established for maintenance treatment of inactive UC. Moreover, recent studies suggest that 5-ASA may also be effective in maintaining remission in Crohn's colitis. The efficacy of 1 year maintenance treatment with oral 4-ASA (1.5 g/d, slow release tablets, *n* = 19) and oral 5-ASA (1.5 g/d, slow release tablets, *n* = 21) was compared in a randomized double-blind trial in patients with quiescent Crohn's ileocolitis. That study concluded that 4-ASA may be as effective as 5-ASA for maintenance treatment of quiescent CD and there were no differences in the severity of relapse between both treatment groups^[18]. In contrast to 5-ASA, no nephrotoxicity related to 4-ASA

has been reported. The only other study that compared these two compounds examined the response to topical treatment in left-sided colitis and showed that 4-ASA and 5-ASA were equally effective^[24].

Mechanism of action of 4-ASA

The anti-inflammatory mechanism by which the aminosalicylates exert their therapeutic action is not well established but several hypotheses have been documented. 5-ASA is a potent inhibitor of arachidonic acid metabolism, decreasing the synthesis of both leukotrienes and prostaglandins. Moreover, 5-ASA is a potent scavenger of free radicals^[25-27].

In contrast, 4-ASA does not seem to have an inhibitory effect on the lipoxygenation of arachidonic acid and is ineffective as a radical scavenger, but it is thought to act via nuclear factor (NF)-κB inhibition^[28]. Both drugs, however, inhibit *in vitro* activation of T and B lymphocytes by pokeweed mitogen in a dose-dependent manner^[29,30]. These findings suggest that the use of either drug in IBD may decrease the heightened state of lamina propria lymphocyte activation as a part of their therapeutic action^[31]. Moreover, mechanisms not affecting arachidonic acid metabolism and superoxide release may contribute to the therapeutic potential of both drugs in IBD^[32].

The most common side effects of 4-ASA include nausea, vomiting, and epigastric pain. Less frequent side effects are fever, joint pains, skin eruptions, and hepatitis; all of which may be attributed to hypersensitivity reactions^[33]. More serious side effects are seen only rarely, such as leukopenia, thrombocytopenia, and agranulocytosis^[33]. Because of the lower incidence of serious side effects, it was thought that delayed release formulation of 4-ASA at higher doses could be used in IBD with higher therapeutic efficacy than seen in previous studies. Schreiber *et al*^[18] concluded that oral 4-ASA may be as effective as 5-ASA for maintenance treatment of remission in CD. Several other studies have suggested that

4-ASA is effective in the treatment of active CD or UC; both as a topical formulation (enema) or as an oral slow-release tablet^[18].

The risk of cross intolerance reaction between 5-ASA and 4-ASA was evaluated by Gisbert *et al*^[34]. They reported three cases of 5-ASA-induced pancreatitis, with no recurrence of pancreatitis during subsequent treatment with 4-ASA enemas. They concluded that 4-ASA enemas are a safe and well-tolerated therapeutic alternative whenever 5-ASA-induced pancreatitis occurs. 4-ASA has been used in the treatment of mild to moderate UC in patients intolerant of sulfasalazine, and in the treatment of CD^[35]. It has been designated an orphan drug by the United States FDA for use in these conditions^[36].

Pharmacokinetics of 4-ASA

4-ASA is readily absorbed from the GIT. It is distributed into various tissues and fluids including peritoneal fluid, pleural fluid and synovial fluid in concentrations approximately equal to plasma concentrations of the drug. Cerebrospinal fluid concentrations of 4-ASA are reported to be 10%-50% of concurrent plasma concentrations of the drug in patients with inflamed meninges. 4-ASA is 50%-73% bound to plasma proteins. The plasma half-life of 4-ASA is about 1 h. Plasma concentrations of the drug are not substantially affected by renal or hepatic insufficiency; however, the half-lives of the inactive metabolites may be prolonged in patients with impaired renal function. 4-ASA is inactivated in the intestinal mucosa and liver primarily by acetylation. The major metabolites are N-acetyl-p-aminosalicylic acid and p-aminosalicylic acid. 4-ASA and its metabolites are excreted in urine by glomerular filtration and tubular secretion. After 2 h in simulated gastric acid, 10% of the dose of unprotected (nonenteric coated) aminosalicylic acid is decarboxylated to form m-aminophenol, a known hepatotoxin^[37].

Dose of 4-ASA

4-ASA is about 50% more potent than 5-ASA, therefore, its therapeutic dose is about 60% of the effective dose of sulfasalazine or 5-ASA on a molar basis. The recommended daily dosage of sulfasalazine is 3-4 g (6-8 tablets) for adults and 40-60 mg/kg/d (divided into 3-4 doses) for children (aged > 6 years). On this basis, a dosage regimen of 0.5-0.75 g of 4-ASA divided into two or three doses can be used^[38].

Contraindications of 4-ASA

4-ASA is contraindicated in patients who are hypersensitive to ASA or who have severe renal diseases, hepatic disease or gastric ulcers^[39].

Interactions of 4-ASA

4-ASA impairs GI absorption of rifampin, reduces rate of acetylation of isoniazid, decreases the GI absorption of digoxin, and enhances the hypoprothrombinemic effect of oral anticoagulants. Concomitant probenecid increases the serum concentration of 4-ASA, ammonium chloride increases probability of crystalluria during therapy,

and diphenhydramine impairs its absorption.

Pregnancy

4-ASA is a FDA pregnancy category C drug.

Adverse effects of 4-ASA

The most frequent adverse effect of 4-ASA is GI disturbance including nausea, vomiting, abdominal pain and diarrhea. The acidic carboxylic group of 4-ASA is responsible for the gastrointestinal (GI) irritation. Occurrence of peptic ulcers and gastric hemorrhage is rare. Adverse GI effect can be minimized in patients by administration of the drug with food or discontinuation of the drug when symptoms are severe. Malabsorption of vitamin B₁₂, folic acid, iron and lipids is also observed. Reported hypersensitive reactions include fever, skin eruptions of various types, pruritis, vasculitis, exfoliative dermatitis, joint pain, eosinophilia, leucopenia, agranulocytosis and thrombocytopenia^[39,40].

COLON-SPECIFIC PRODRUGS OF 4-ASA

Extensive clinical studies have shown the effectiveness and safety of 4-ASA for the topical treatment of active ulcerative proctitis or left-sided UC^[23]. It presents many advantages over 5-ASA, such as: more stability, more potency, and absence of incidences of pancreatitis, but shares the same drawback of fast and extensive absorption in the upper GIT, before it reaches the colon. This is due to its weakly acidic nature (pKa 3-4), which results in its ready absorption in the upper GIT^[16]. Studies by Selby suggest that 4-ASA is a stable, inexpensive alternative to 5-ASA for the topical treatment of UC or for linking to carrier molecules for release in the colon^[24].

Many colon-specific prodrugs of 5-ASA have been cited in the literature, while the prodrug approach has been only sporadically applied for colonic delivery of 4-ASA, for reasons that are not known. The possible reason could be the steric problem of 4-ASA, due to which it cannot be linked with same polymers (*e.g.*, dextran, chitin, hydroxymethyl cellulose or synthetic polymers) as 5-ASA or to other polymers (*e.g.*, cyclodextrins) using the same methods that have been used for 5-ASA^[20].

Zhao *et al*^[41] were the first to design a colon-targeting amide prodrug of 4-ASA (Figure 1D) with the nonessential amino acid glycine for the treatment of IBD. The amide prodrug was synthesized by reacting amino and hydroxyl protected 4-ASA acyl chloride with glycine, followed by deprotection. *In vivo* experiments on rats suggested that 4-aminosalicylglycine showed more curative effect than 4-ASA.

Wallace *et al*^[42] have patented L-arginine salts of 4-ASA and 5-ASA (Figure 1E) for inflammatory conditions of the GIT, and for irritable bowel syndrome (IBS). They claim that the salt is more effective in reducing the inflammation in the colon compared to individual drugs or L-arginine, as demonstrated from overall greater reduction in the colitis-associated edema, granulocyte infiltration, and body weight loss with enhanced free-radical scavenging and more potent antioxidant activity, thus

providing a synergistic effect. They ascribe the synergistic effect to the ability of arginine to act as a source of nitrogen for NO, which is a potent vasodilator that attenuates intestinal tissue damage^[43,44].

A US Patent describes 5-ASA and 4-ASA prodrugs as having an H₂S-releasing moiety linked via an azo, ester, anhydride, thioester or amide linkage (Figure 1F) for the treatment of IBD and IBS and chemoprevention of colon cancer^[45]. The basis for using H₂S-releasing carriers in this design is their encouraging and beneficial pharmacological profile. There is documented evidence for antinociceptive effect of H₂S in the GIT by activating K_{ATP} channels and smooth muscle relaxant activity in intestinal tissue^[46].

Based on the design of sulfasalazine and using azo bond chemistry, Zhao *et al*^[47] have reported the synthesis of prodrugs of 4-ASA with salicylic acid, hydroxybenzene and N-salicyloyl glycine (Figure 1G), aiming to target 4-ASA to the colon for management of IBD with enhanced efficacy and fewer side effects. However, we could not find any reports of activity of these prodrugs in any of the experimental models of IBD.

Oxidative stress is one of the key players in the pathogenesis of IBD causing cellular injury and colonic inflammation^[48]. Keeping this in mind, Sheng *et al*^[49] designed azo-linked colon-specific prodrugs of 4-ASA (Figure 1H) with several substituted phenols such as m-nitrophenol, salicylamide, o-chlorophenol, m-methylphenol, o-methylphenol, o-nitrophenol and o-dihydroxybenzene, possessing antioxidant properties. Their main aim was to achieve co-antioxidant effects of 4-ASA and substituted phenol that would result in higher reducibility and synergistic free-radical scavenging. These phenols were chosen on the basis of their reducibility, electronic and steric factors. 4-(3',4'-dihydroxyphenyl) azobenzene-2-acetylsalicylic acid, 4-(2'-methyl-4'-hydroxyphenyl) azobenzene salicylic acid and 4-(3'-methyl-4'-hydroxyphenyl) azobenzene salicylic acid were reported to be the compounds with maximum anti-inflammatory and antioxidant activities.

Dhaneshwar *et al*^[12] have explored mutual amide prodrug strategy for 5-ASA with several amino acids. Applying the same concept further to 4-ASA, they have reported its amide conjugates with D-phenylalanine (Figure 1I) and L-tryptophan (Figure 1G). The proposed mechanism of activation was enzymatic by the action of N-acyl amidases secreted by colonic bacteria. The amino acids used in the design were nontoxic carriers like D-phenylalanine and L-tryptophan, possessing wound healing and anti-inflammatory activities. The prodrugs furnished 86%-91% release of 4-ASA in rat fecal matter in 20 h. Their curative effect was investigated in a 2,4,6-trinitrobenzene-sulfonic-acid-induced experimental colitis model in rats. Pancreas, liver and stomach of rats were studied by histopathology for the assessment of any adverse reactions. The prodrugs were found to possess significantly superior safety profile than sulfasalazine, oral 4-ASA and 5-ASA, with comparable efficacy to sulfasalazine.

In an innovative attempt, colon-specific 4-ASA-D-

glucosamine amide prodrug (Figure 1K) was developed by Dhaneshwar *et al*^[50] out of the urgent need to search for such alternate options that would not produce 5-ASA-induced pancreatitis and sulfapyridine-induced hepatitis. The hypothesis was to offer supplementation of D-glucosamine that would suppress activation of intestinal epithelial cells, thus helping to maintain the integrity of the colonic architecture. The protective effect of this prodrug was compared with 5-ASA-D-glucosamine conjugate as well as standard drugs like sulfasalazine, 4-ASA and 5-ASA on the course of 2,4,6-trinitrobenzene sulphonic acid (TNBS)-induced colitis. The mitigating effect of 5-ASA prodrug was comparable to that of sulfasalazine, while that for 4-ASA prodrug was moderate. However, hepatitis or pancreatitis was not seen with 4-ASA prodrug^[50].

A polymeric prodrug of 4-ASA (Figure 1L) with an acrylic polymeric system and degradable ester bonds, such as hydroxy propyl methacrylate, was synthesized and evaluated for colon-targeted drug delivery by Yadav *et al*^[51]. In the first 2 h, 40.01% release was observed in rat fecal matter at pH 7.4, which was followed by sustained release of 93% over a period of 12 h. The authors propose that the developed delivery system could be useful for controlled release, prolonged transit time, and colon-targeted delivery of 4-ASA.

A macromolecular colon-targeting ester prodrug of 4-ASA with β -cyclodextrin as a promoiety was reported very recently by Vadnerkar *et al*^[52]. Interestingly, 20%-23% release of 4-ASA was observed in the stomach and small intestinal homogenates, while the prodrug was found to be resistant to pH-dependent hydrolysis at pH = 1.2 and 7.4. Almost 68% and 92% release was obtained in rat cecal and fecal matter, respectively. The prodrug demonstrated a moderate ameliorating effect on TNBS-induced colitis as compared to sulfasalazine or 4/5-ASA administered rectally, but it was similar to that with orally administered aminosalicylates. Partial activation of the prodrug in the upper GIT homogenates causing incomplete transport of 4-ASA to the site of action could be responsible for its moderate effect. However, the prodrug was reported to be safe, producing no harmful effects on the stomach, liver or pancreas of rats^[52].

In another innovation, Suneela *et al*^[53] designed four azo prodrugs of 4- and 5-ASA using the same aminosalicylates as carriers. As known already, olsalazine is a dimer of 5-ASA, which is synthesized by coupling diazo salt of 5-ASA with salicylic acid, which upon reduction by azo reductase, generates two molecules of 5-ASA. However, the azo prodrugs reported by Suneela *et al*^[53] are different in the sense that they are prepared by coupling diazo salt of either 4-ASA or 5-ASA with 4-ASA or 5-ASA as carriers in all possible permutations and combinations. This results in prodrugs that, upon activation by azo reductase reduction, generate only one molecule of either 4-ASA or 5-ASA, while the other molecule released is a diaminosalicylic acid and not another molecule of aminosalicylate. These prodrugs were designed to target the colon affected with IBD. Due to their improved hydrophilic nature, the prodrugs had minimum absorption in the

upper GIT. The prodrugs were considerably stable when incubated in the upper GIT. Approximately 68%-91% release was obtained on incubation with rat cecal matter. Amongst the series of four prodrugs, the prodrug of 4-ASA with 4-ASA (4A-4AAz) (Figure 1M) at a dose of 53 mg/kg was found to alleviate all the quantifying markers of TNBS-induced experimental colitis in Wistar rats. Due to absence of adverse effects in the stomach, liver and pancreas, it could be a promising alternative for IBD patients intolerant to 5-ASA-induced pancreatitis and sulfapyridine-induced adverse effects observed with sulfasalazine^[53].

CONCLUSION

Despite possessing more stability and potency than 5-ASA in treating UC, without the evident risk of 5-ASA-induced pancreatitis, 4-ASA or its colon-targeted prodrugs remain unexplored and neglected when it comes to the management of IBD patients intolerant to 5-ASA. There is a need to investigate this promising compound, explore its untapped potential, and develop safe but effective systems for its targeted delivery to the colon, for better management of IBD as well as other local diseases of colon like IBS and colorectal cancer.

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Anti-TNF alpha in the treatment of ulcerative colitis: A valid approach for organ-sparing or an expensive option to delay surgery?

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Abstract

Ulcerative colitis (UC) is an inflammatory bowel disease affecting large bowel with variable clinical course. The history of disease has been modified by the introduction of biologic therapy, in particular Infliximab (IFX), that has demonstrated efficacy in inducing fast symptoms remission, promoting mucosal healing and maintaining long-term remission. However, surgery is still needed for UC patients: in case of failure of medical therapy and if acute complications or a malignancy occurred. Surgical treatment is associated with a short-term post-operative mortality and morbidity respectively of 0%-4% and 30%. In this study we systematically analyzed: the role of IFX in reducing the colectomy rate, the risk of post-operative morbidity in pre-operatively IFX-treated patients and the cost-effectiveness of IFX therapy. Four of 5 analyzed randomized controlled

trials demonstrated that therapy with IFX significantly reduces the colectomy rate. Moreover, pre-operative treatment with IFX doesn't seem to increase post-operative infectious complications. By an economic point of view, the cost-effectiveness of IFX-therapy was demonstrated for UC patients suffering from moderate to severe UC in a study based on a cost estimation of the National Health Service of England and Wales. However, the argument is debated.

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Key words: Ulcerative colitis; Infliximab; Colectomy; Post-operative complications; Cost-effectiveness; Inflammatory bowel disease

Core tip: The introduction of biologic therapy with Infliximab (IFX) has significantly modified the clinical course of ulcerative colitis. In this study we systematically analyzed the role of IFX in reducing the colectomy rate, the risk of post-operative morbidity in pre-operatively IFX-treated patients and the cost-effectiveness of IFX therapy.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease, of an unknown etiology, affecting the large bowel. It is characterized by a contiguous mucosal inflammation

starting in the rectum and proximally progressing in continuity in the colon for a different distance. According to the Montreal Classification, which describes the maximal macroscopic extent of the disease at colonoscopy, the distribution of UC is commonly classified as: proctitis, left-sided and extensive colitis^[1-4]. Disease activity is grouped into remission, mild, moderate, or severe but the clinical course of UC is variable and can range from a long-standing remitting to a refractory or fulminant disease^[5-11]. Solberg *et al.*^[5], in a population-based cohort study of 843 patients with inflammatory bowel disease, enrolled in South-Eastern Norway and systematically followed-up at 1, 5 and 10 years after diagnosis, identified 4 different clinical patterns: (1) initial high activity to remission or mild severity (55%); (2) chronic intermittent symptoms (37%); (3) continuous symptoms (6%); and (4) initial low activity to increased severity (1%)^[12]. The main symptoms of UC are bloody diarrhea and abdominal pain, associated with urgency and tenesmus. UC is conventionally treated with a step-up approach, based on the severity and extent of the disease, including various agents such as 5-aminosalicylates, corticosteroids and immunosuppressants (including thiopurines and cyclosporine). The primary aims of medical therapy in UC should be inducing and maintaining long-term remission, achieving mucosal healing, minimizing steroid-dependence, avoiding serious complications (hospitalization and surgery) and improving patients' quality of life^[13-17]. However, standard therapy is not always able to achieve these goals and patients become steroid-dependent or experience frequent or severe relapse, with consequent increased risk of hospitalization and surgery. The history of disease has been partially modified by the introduction of biologic therapies. Infliximab (IFX) has demonstrated efficacy in inducing fast symptoms-remission, promoting mucosal healing and maintaining long-term remission^[17-20]. It is currently approved for patients with moderate to severe UC who have incomplete response, are intolerant or have any medical contraindications to corticosteroids and/or immunomodulators^[21-24]. It is also recommended as rescue therapy in severe steroid-refractory disease^[25-27] and in steroid-dependent patients^[28,29].

Surgery is still needed for UC patients, in case of failure of medical therapy, occurrence of acute complications (such as fulminant colitis, toxic megacolon and bowel perforation) or development of malignancy. Since its first description (1978), restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) represents the gold standard of surgical treatment of UC: all the colon and rectum are removed and a J pouch is created with terminal ileum and anatomized to the anal canal. This restorative operation, avoiding a permanent stoma, maintains intestinal continuity and preserves the patient's body image^[30-33]. Moreover, the introduction of minimally invasive approach significantly contributed in improving the acceptance and tolerability of this procedure^[34-37], reducing the rate of post-operative adhesions and post-operative hospital stay and improving cosmetic results^[38-41].

However, even if in skilled hands, proctocolectomy with IPAA is not without risk and is associated with an estimated short-term mortality ranged between 0% and 4% and a morbidity rate of about 30%, with an incidence of pelvic sepsis ranging from 5% to 24% and a re-surgery risk of about 16%^[42,43].

If surgery represents a definitive solution for cessation of symptoms, withdrawing medical therapies and reducing the cancer-risk, it is not free of long-term post-operative morbidity (as pouchitis, fecal incontinence, female fecundity or fertility) with a relevant impact on patients' quality of life^[44-46]. Population-based studies have reported a 10-years cumulative risk of colectomy ranging between 9%-30%, with some differences among countries. Approximately, 4% to 9% of UC patients will require colectomy within the first year of diagnosis and, subsequently, the risk of colectomy increases of 1% per year^[47-50].

It is still under debate whether, in the long-term, the biological therapies could be a valid approach for organ-sparing, rather than an expensive option to delay surgery. Aim of this review was to evaluate the real impact of biological therapy on the rate of colectomy in UC patients.

A review of the literature searching for the terms "anti-tumor necrosis factor- α (TNF- α)", "infliximab" matched with the terms "ulcerative colitis" and "surgery" was performed, using PubMed, MEDLINE, EMBASE and Cochrane databases. All relevant articles (both experimental and observational studies) in English between January 2000 and July 2013 were reviewed.

Therapy with infliximab and rate of colectomy

We identified five randomized controlled trials^[51-54], one meta-analysis^[55] and six observational studies^[56-61] following literature search. Characteristics of the studies are summarized in Table 1.

RANDOMIZED CONTROLLED TRIALS AND META-ANALYSIS

Two randomized, double-blind, placebo-controlled trials, ACT 1 and ACT 2, demonstrated the efficacy of IFX for induction (week 8) and maintenance (week 30 and week 54 for ACT1) of clinical response and remission in patients with moderate-to-severe UC, despite the use of conventional therapy^[51]. Further analysis, from ACT 1-2 open-label extension phase, focused on colectomy and hospitalization rates during follow-up to 54 wk. Compared with placebo, the cumulative incidence of colectomy through 54 wk for IFX was significantly lower (10% *vs* 17%, $P = 0.02$), with an absolute risk reduction of 7% (95%CI: 0.01-0.12, HR = 0.59). Moreover, in IFX-treated patients were recorded fewer (compared to placebo group) UC-related hospitalizations and surgical procedures per 100 patient-years of treatment (40 *vs* 20, $P = 0.003$; 34 *vs* 21, $P = 0.03$ respectively)^[52].

Previous controlled smaller studies have addressed the risk of colectomy in patients with severe UC treated

Table 1 Rate of colectomy after therapy with Infliximab

Ref.	RCT	Pts (n)	Type of disease	FU	Rate of colectomy in IFX Pts	Rate of colectomy in control Pts	P value
Rutgeerts <i>et al</i> ^[51]	Y	364 (IFX)	Moderate to severe UC	54 wk (ACT1)	9.5%	14.7%	< 0.05
Sandborn <i>et al</i> ^[52]		364 (control)		30 wk (ACT2)			
Sands <i>et al</i> ^[53]	Y	8 (IFX)	Severe active steroid-refractory	2 wk	50%	100%	NS
		3 (control)					
Järnerot <i>et al</i> ^[25]	Y	24 (IFX)	Severe to moderate UC not responding to conventional therapy	3 mo	29.2%	66.7%	0.017
		21 (control)					
Gustavsson <i>et al</i> ^[54]	Y	24 (IFX)	Severe to moderate UC not responding to conventional therapy	36 mo	50%	76%	0.012
		21 (control)					
Aratari <i>et al</i> ^[56]	N	15 (IFX)	Severe steroid-refractory UC	26 mo	18%		
Teisner <i>et al</i> ^[57]	N	52 (IFX)	Acute, severe and chronic refractory UC	22 mo	27%		
Ferrante <i>et al</i> ^[58]	N	121 (IFX)	Acute severe refractory UC	33 mo	17%	-	-
Oussalah <i>et al</i> ^[59]	N	191 (IFX)	UC	18 mo	18.8%	-	-
		Multicenter					
Desmond <i>et al</i> ^[60]	N	21 (IFX)	UC	14 mo	9.5%	-	-
Garcia-Planella <i>et al</i> ^[61]	N	22 (IFX)	UC	84 mo	27.3%	-	-

RCT: Randomized controlled trial; Pts: Patients; FU: Follow-up; IFX: Infliximab; UC: Ulcerative colitis; Y: Yes; N: Not.

with IFX as rescue therapy. In 2001 Sands reported data on 11 patients with severe steroid-refractory disease, of whom 8 treated with IFX and 3 with placebo. After 2 wk, all patients treated with placebo underwent to surgery, while only 50% of patients receiving IFX needed surgery; however, the sample size was too small to detect a statistically significant benefit^[53]. Later, 45 patients with moderate to severe UC were randomized to IFX or placebo (24 *vs* 21 respectively) both after four day from the start of corticosteroid treatment. In the placebo group more patients (14/21, 66.7%) than IFX group (7/24, 29.2%) had a colectomy ($P = 0.017$; OR = 4.9; 95%CI: 1.4-17) within 3 mo after randomization^[25]. After a follow-up of 3 years, 50% of patients in the IFX group and 76% in the placebo group had a colectomy ($P = 0.012$)^[54].

Recently, Costa *et al*^[55] presented data from a meta-analysis on the benefit of IFX in reducing hospitalization and/or major surgeries in patients with inflammatory bowel disease. They analyzed 11 studies: 5 randomized controlled trials (RCTs) and 6 observational studies. In the RCTs, IFX treatment was associated with a significant 43% odds reduction of overall major surgery risk (OR = 0.57; 95%CI: 0.37-0.88) with a number-to-treat to avoid colectomy of 11 (95%CI: 6-51) for 1.2 years. However, a not significant increase was found in pooled results from observational studies (OR = 1.43; 95%CI: 0.65-3.13). The authors concluded that this discrepancy could be explained by the heterogeneity of observational studies, including patients at high risk of colectomy due to more severe disease and refractoriness to previous treatment.

OBSERVATIONAL STUDY

The first data on the long-term risk of colectomy were reported in a study of 314 UC patients from Italy. Among them, 52 (16.5%) patients had severe UC and were treated with intravenous corticosteroids for a median of 7 days. Of 15 patients who did not respond,

11 received IFX with short-term clinical benefit and 4 underwent urgent colectomy. In the long-term follow-up, another 6 patients underwent elective colectomy for a disease relapse, with a total colectomy rate, following the acute flare-up, of 19%. The long-term colectomy risk was not different between patients treated with IFX and steroid-responsive patients (18% *vs* 11%, respectively), as IFX was able to avoid urgent colectomy, but not to reduce the risk of elective surgery^[56]. The risk of long-term colectomy in severe UC was also evaluated in a smaller Danish study of 52 UC patients. Nineteen (37%) patients had severe UC and 7 of them (37%) underwent colectomy after a median follow-up of 22 mo (range 4-57 mo). Among the remaining patients with a chronic refractory UC, the colectomy rate was 21%. The authors concluded that IFX can avoid colectomy in two-thirds of the patients with acute, severe UC, but the beneficial effect on colectomy rate in chronic, refractory UC seems less convincing^[57]. Long-term data on colectomy in UC patients treated with IFX come from referral centers studies. In the Leuven's cohort of 121 refractory UC patients (patients with acute severe attack, refractory to intravenous steroids were excluded), 21 patients (17%) came to colectomy and 68% of initial responders achieved a sustained clinical response during a median follow-up of 33 mo (IQR 17-49.8). Lack of short-term clinical benefit, high values of baseline C-reactive protein (CRP) and previous intravenous steroid or cyclosporine treatment were identified as independent predictors of colectomy^[58]. These results are similar to those reported in a French multicenter study, in which, among 191 patients who received at least one IFX infusion, 36 patients (18.8%) underwent colectomy during a median follow-up of 18 mo (IQR 8-32). Independent predictors of colectomy were: no clinical response after IFX induction, high baseline CRP value, previous treatment with cyclosporine and IFX indication for acute severe UC^[59]. Furthermore, other experience supported history of hospital admission as an indepen-

Table 2 Rate of overall post-operative morbidity and post-operative infectious complications in patients pre-operatively treated with Infliximab

Ref.	RCT	Pts (IFX vs control) (n)	PO morbidity (IFX vs control)	P value	PO infectious complications (IFX vs control)	P value
Schluender <i>et al</i> ^[64]	N	17 vs 134	36% vs 28%	> 0.05	18% vs 8%	> 0.05
Selvasekar <i>et al</i> ^[62]	N	47 vs 254	62% vs 49%	0.10	28% vs 10%	< 0.01
Mor <i>et al</i> ^[63]	N	46 vs 46	34.8% vs 15.2%	0.004	21.7% vs 2.2%	0.011
Ferrante <i>et al</i> ^[65]	N	22 vs 119	11.1% vs 28.6%	> 0.05	9% vs 24%	0.161
Gainsbury <i>et al</i> ^[66]	N	29 vs 52	44.8% vs 44.2%	0.96	17.2% vs 26.9%	0.32
Rizzo <i>et al</i> ^[67]	N	16 vs 22	37.5% vs 22.7%	> 0.05	18.7% vs 18.2%	> 0.05

RCT: Randomized controlled trial; Pts: Patients; PO: Post-operative; IFX: Infliximab; Y: Yes.

dent predictor of the need of colectomy^[60,61].

Peri-operative infliximab and post-operative outcome

An increasing number of patients undergo to surgery after experienced biologic therapy. There is an emerging concern on the safety profile of IFX in the peri-operative setting in potentially pre-surgical patients. Many groups have reported their experiences for UC patients and there is not an agreement on the impact of these drugs on post-operative complications^[62-67]. The main characteristics of the studies are summarized in Table 2. Recently, Yang *et al*^[68] performed a high quality meta-analysis based on five studies, including 706 patients, who were treated with IFX before restorative procto-colectomy with IPAA. The authors did not find a strong association between pre-operative treatment with IFX and short-term infectious complications (OR = 2.24), but it was associated with a significantly increased risk of short-term overall post-operative complications (OR = 1.80). However, these results need to be interpreted with caution. The subgroup analysis was underpowered to assess the nature of these complications because of the small sample size and heterogeneity of the included studies (end-points, patients' characteristics and indication, type and timing of surgery).

Infliximab and surgery: Cost-effectiveness

An important issue of IFX therapy is its cost-effectiveness. IFX is often perceived to be an expensive treatment option for patients with IBD. Tsai *et al*^[69] made a cost-effectiveness analysis in UC patients based on a cost estimation of the National Health Service of England and Wales for the year 2006-07. At analysis of responders patients only, therapy with IFX was associated at an additional 0.753 quality-adjusted life year (QALYs) at an additional cost of £20662 compared to standard care without IFX; the estimated incremental cost per QALY gained for IFX against standard care was £27424. At analysis of remission patients, therapy with IFX derived an additional 0.387 QALYs at an additional cost of £7615 compared with standard care without IFX. The estimated incremental cost per QALY gained for IFX against standard care was £19696. The authors conclude that therapy with IFX appears to be a cost-effective treatment option for

adult patients suffering from moderate to severe UC. In a recent study, Park *et al*^[70] created a Markov model simulating 2 cohorts of 21-year-old patients with severe UC, following them until 100 years of age or death, comparing early colectomy with IPAA strategy to the standard medical therapy strategy (including IFX). In this study standard medical therapy accrued a discounted lifetime cost of \$236370 per patient; in contrast, early colectomy with IPAA accrued a discounted lifetime cost of \$147763 per patient. QALY-gained for standard medical therapy was 20.78, while QALY-gained for early colectomy with IPAA was 20.72; the resulting incremental cost-effectiveness ratio was approximately \$1.5 million per QALY-gained. So, the authors concluded that early colectomy with IPAA after diagnosis of severe UC reduce health care expenditures and provides comparable quality of life compared exhaustive standard medical therapy. Only an extremely low quality of life after IPAA could maintain the standard medical therapy strategy as the optimal management strategy in severe UC.

CONCLUSION

IFX has demonstrated efficacy in inducing and maintaining clinical and endoscopic remission in the long run. IFX can also avoid urgent colectomy in patients with severe acute UC refractory to intravenous steroids. The real impact of biological therapy on the natural history of UC is still controversial, whereas it is not clear if it allows avoidance of colectomy or rather than a delay. The median colectomy risk for UC patients treated with IFX is about 10%-20% in both RCTs and observational studies, with higher rate for patients with severe acute attack. Data from RCTs support the efficacy of IFX in reducing the risk of surgeries in the long-term, but none was designed to assess IFX effects on surgeries. Real life data from referral centers do not confirm this issue, but each study includes patients with different baseline characteristics and risks of colectomy. From these evidences, it seems that patients with more severe disease, high inflammation burden, refractoriness to intravenous steroids and/or cyclosporine and history of hospitalizations, have higher risk of colectomy. So, it is necessary for physicians taking in account the risks and the benefits of medical

versus surgical therapy, concerning about cost and side effects of medications, cost and morbidity of surgery and patients' quality of life. Prospective, specifically designed studies are necessary to assess the long-term risk of colectomy in UC patients treated with IFX.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Inflammatory bowel disease and celiac disease: Overlaps and differences

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the specific target organ, the bowel. The etiology and immunopathogenesis of both conditions characterized by chronic intestinal inflammation, inflammatory bowel disease (IBD) and celiac disease (CeD), are not completely understood. Both are complex diseases with genetics and environment contributing to dysregulation of innate and adaptive immune responses, leading to chronic inflammation and disease. CeD constitutes a particular disease because the main environmental and genetic triggers are largely known. IBD comprises two main clinical forms, Crohn's disease and ulcerative colitis, which most likely involve a complex interplay between some components of the commensal microbiota and other environmental factors in their origin. These multifactorial diseases encompass a broad spectrum of clinical phenotypes and ages of onset, although the clinical presentation often differs depending on childhood or adult onset, with greater heterogeneity commonly observed in adults.

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Key words: Disease susceptibility; Gene-environment interaction; Immune system; Inflammation; Microbiota; Inflammatory bowel disease

Core tip: Inflammatory bowel disease and celiac disease are two immune-mediated diseases characterized by chronic intestinal inflammation. Recent findings demonstrate shared genetics and functional pathways. We reviewed the extension of these overlaps to other disease features and suggest future research approaches.

Abstract

Recent findings demonstrate the common genetic basis for many immune-mediated diseases, and consequently, the partially shared pathogenesis. We collected these findings and reviewed the extension of these overlaps to other disease characteristics. Two autoimmune diseases were selected that also share

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INTRODUCTION

The immune system is essential for defense self against external pathogens, and to maintain homeostasis. Dysregulation between those two processes contributes to development of immune-mediated diseases. These diseases represent an important cause of chronic illness, with a consequent high impact on public health. Different diseases can be found under this term, including autoimmune and inflammatory conditions. In these cases, it is common to find a specific organ affected, as occurs in inflammatory bowel disease (IBD) and celiac disease (CeD), both involving damage of the gastrointestinal tract. The gut is highly exposed to exogenous and endogenous antigens and controlled inflammation is a key process in maintaining homeostasis. Different factors can contribute to alter this equilibrium and to disrupt health status. Decades of research have focused on identifying those contributing factors and the reason why one specific disease develops. Recent findings demonstrate the extensive overlap in the genetic basis of immune-mediated diseases, including IBD and CeD.

The present review summarizes the current knowledge of different features related to the two major clinical forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC), and CeD, paying special attention to the overlaps and differences between them. These two diseases share genetic risk factors, and it would be interesting to know whether this overlap also extend to other disease characteristics in order to gain knowledge about common pathogenic mechanisms and possible shared treatments.

CeD is one of the most frequent immune-mediated diseases. In Europe and the US the current prevalence of CeD is around 1 per 100 individuals^[1,2] and a similar prevalence probably exists worldwide, although it has not been as extensively studied^[3,4]. IBD shows a lower prevalence with values ranging from 26 to 199 per 100000 individuals in CD and from 37 to 246 per 100000 individuals in UC^[5]. The prevalence of IBD is higher in developed countries and urban areas. A recent increase in the prevalence of both CeD and IBD has been described as a consequence of several factors^[6-8]. In CeD these factors include the development of more effective diagnostic tools^[9].

IBD affects both sexes similarly and the highest incidence is found between the second and the fourth decade of life. CeD seems to be more frequent in women^[10], although this depends on the age at onset^[11]. CeD can be diagnosed in individuals at any age, but it appears more frequently during childhood^[12].

The prevalence of CeD in patients with IBD is not clear. There are several cases in the literature describing the coexistence of both diseases in the same family or even in the same patient^[13-18]. However, some authors consider that this is an incidental association and the

prevalence of CeD is similar between IBD patients and the general population^[19]. In fact, CD and UC patients are not considered a risk group for routine CeD screening.

CLINICAL PRESENTATION

A wide spectrum of clinical symptoms characterizes IBD and CeD. In addition, differences in the clinical presentation can be found depending on the age at diagnosis.

IBD manifests during childhood or adolescence in at least 20% of patients. This presentation is commonly more severe and extensive than that observed in adult-onset disease^[20]. In CD, involvement of the upper gastrointestinal tract is more frequently observed at early onset^[21]. Bloody, mucous diarrhea is the almost universal hallmark of UC, although additional symptoms may be also present. The initial symptoms of CD are more subtle and varied, partly as a result of its diffuse and diverse anatomical location. The constellation of abdominal pain, diarrhea, poor appetite and weight loss constitutes the classical presentation of CD in all age groups, and is the mode of presentation in nearly 80% of children and adolescents (with or without extraintestinal manifestations). Abdominal pain is the most common single symptom at presentation^[22,23].

In CeD, the presentation may be variable, but diarrhea, which may be acute or insidious in onset, is the most common presenting symptom in children. On the contrary, mild and nonspecific gastrointestinal symptoms are common in adults with CeD, with intermittent diarrhea, diffuse abdominal pain, dyspepsia, constipation, asthenia, flatulence, bloating or abdominal discomfort being the most frequently observed symptoms. In adults, iron-deficiency anemia without response to any appropriate treatment is a frequently observed sign^[24,25].

Extraintestinal manifestations may be present in IBD and CeD. In IBD, most of the extraintestinal manifestations are shared by CD and UC. They may accompany intestinal symptoms or, less commonly, precede or overshadow them. These manifestations seem to be related to activity (relapse/remission) and location of the disease. Although children and adults may share extraintestinal manifestations, their frequency is usually different. Ocular lesions seem to be less common in young patients and a childhood-onset of IBD, particularly CD, may represent a specific risk factor for long-term morbidity from clinical osteoporosis^[26].

In CeD, extraintestinal manifestations are usually a consequence of nutrient malabsorption and may coexist with digestive symptoms. It is currently accepted that extraintestinal signs and symptoms are common and may be the only presenting manifestation, mainly in adults^[25,27].

Table 1 summarizes the main clinical features for IBD and CeD. Several overlapping characteristics can be observed. Diarrhea and abdominal pain are digestive symptoms commonly observed in both groups, but they also share several extraintestinal manifestations, as iron-deficiency anemia, short stature or osteoporosis. Some

Table 1 Main clinical features associated with inflammatory bowel disease and celiac disease

IBD	CeD
Intestinal mucosal involvement	Intestinal mucosal involvement
Clinical heterogeneity	Clinical heterogeneity
Depending on location and severity	Depending on degree of gluten sensitivity and amount of gluten ingested
Symptomatic (relapses/remission)	Commonly symptomatic (early onset)
	Mono or oligosymptomatic (late onset)
Digestive signs or symptoms	Digestive signs or symptoms
Diarrhea (\pm rectorrhagia)	Diarrhea
Abdominal pain (less predominant in UC)	Abdominal distension
	Abdominal pain
	Constipation
	Dyspepsia
	Recurrent vomiting
	Pyrosis and regurgitation
	Irritable bowel syndrome with diarrhea predominance
Extraintestinal manifestations	Extraintestinal manifestations
Refractory iron-deficiency anemia	Refractory iron-deficiency anemia
Short stature	Short stature
Poor appetite	Failure to thrive
Weight loss (less prevalent and extreme in UC)	Dermatitis herpetiformis
Sexual maturation delay	Vitamin B12 deficiency
Pneumopathies	Neurological symptoms
Psychological syndromes	Menstrual disturbances
Joints: arthritis and arthralgias (the most common in both CD and UC)	Bleeding diathesis (malabsorption of vitamin K)
Ocular: acute episcleritis, uveitis, orbital myositis	Paresthesia, cramps and tetany (hypocalcemia)
Skin: erythema nodosum, pyoderma gangrenosum	Hepatobiliary system: hypertransaminasemia
Hepatobiliary system: primary sclerosing cholangitis (less predominant in CD), autoimmune hepatitis (unusual)	Osteopenia, osteomalacia and osteoporosis
Renal system: ureteral obstruction, hydronephrosis, urinary stones	Edema, ascites and anasarca (hypoproteinemia)
Vascular system: thrombocytosis, hyperfibrinogenemia, elevated factor V-VII, depression antithrombin III	Hypopituitarism and adrenal insufficiency
Bone: osteoporosis (less predominant in UC)	Recurrent mouth ulcers
Severe complications	Severe complications
Malnutrition with weight loss and emaciation	(In refractory CeD or in patients who do not follow a GFD)
Fistulae	Collagenous CeD
Abscesses	Ulcerative jejunitis
Obstruction	T cell lymphomas
Perforation	
Dysplasia and colorectal cancer	

IBD: Inflammatory bowel disease; CeD: Celiac disease; UC: Ulcerative colitis; CD: Crohn's disease; GFD: Gluten-free diet.

differences are also observed. CeD patients can remain asymptomatic, in contrast with the symptomatic IBD patients.

The major complications of CeD, ulcerative jejunitis and intestinal lymphoma, cause more severe clinical manifestations that may resemble CD, such as acute and persistent abdominal pain, weight loss, signs of intestinal obstruction or gastrointestinal bleeding, fever or signs of marked malnutrition^[28]. It has been suggested that complicated CeD should be considered in CD patients who do not respond to immunosuppressive or biological treatments^[29].

SEROLOGY

As frequently observed in autoimmune diseases, CeD is characterized by the presence of autoantibodies, which are currently included in the definition and diagnostic guidelines for CeD^[30]. Transglutaminase type 2 (TG2) is the major autoantigen in CeD and the target antigen for

endomysial antibodies (EMA) and anti-TG2 antibodies. Therefore, those two antibodies are the most specific for CeD diagnosis. Although a high correlation exists between anti-TG2 and EMA antibodies, the highest specificity is observed for EMA, because anti-TG2 can be present in individuals with other conditions, including CD and UC. However, it is difficult to know the frequency of anti-TG2 antibodies in IBD patients because the studies developed with that aim have yielded different results, probably partially caused by the wide variety of commercial kits used^[18,19,31-35].

On the contrary, the presence of specific antibodies is not a common feature in IBD. Two major groups of serological markers have been described in these patients: those against microbial antigens and autoantibodies. Their relevance for IBD diagnosis is not as strong as for CeD, but they are useful to differentiate CD from UC patients. Among the antibodies against microbial agents, those against *Saccharomyces cerevisiae* (ASCAs) are the most extensively studied and they are related to CD. These anti-

bodies have also been described in CeD patients but they disappear after taking a gluten-free diet (GFD), which is supposedly due to the association of ASCAs with inflammation of the small bowel, and therefore, questions their specificity for CD^[36]. Regarding autoantibodies, anti-neutrophil cytoplasmic antibodies have a high prevalence in UC^[37].

ETIOLOGY

The causes underlying the development of IBD and CeD have not been completely unraveled, but both diseases show a multifactorial origin with a complex genetic and environmental involvement.

Environment

In this regard, CeD is the best-understood immune-mediated disease because the main environmental factor involved is largely known. CeD is triggered by ingestion of dietary wheat gluten or analogous proteins present in other cereal grains, mainly rye and barley.

Although gluten intake is necessary to develop CeD, other environmental factors may play a role. Infections have been related to CeD development. Specifically, rotavirus^[38] and hepatitis B and C virus infections have been observed in CeD patients^[39]. A protector role for breastfeeding at the moment of gluten introduction has also been described^[40].

Viruses have also been implicated in the origin of IBD. The hygiene hypothesis establishes that lack of early exposure to microbial agents due to severe hygienic conditions could increase the likelihood of developing autoimmune and allergic disorders, and it has been used to explain the rising prevalence of IBD observed in industrialized countries^[41]. Although to a lesser extent, this hypothesis has also been proposed for CeD^[42]. Recent genetic findings support a role of pathogens in IBD and CeD.

Other environmental factors contributing to IBD risk are smoking and appendectomy. The effect of cigarette smoking is opposite in both forms of IBD: beneficial in UC and harmful in CD^[43,44]. For appendectomy, it seems that it reduces the risk of UC^[45,46].

Vitamin D levels, diet, hormone use and stress have also been postulated as risk factors for one or both main forms of IBD, but they need to be further investigated^[47].

The environmental influence in IBD pathogenesis seems to involve complex mechanisms because its role in disease risk may be modified by other factors such as sex, geographic region, or genetic background^[47]. Moreover, environmental factors are probably influencing the natural history in addition to the origin of these diseases.

Microbiota

An altered microbiota composition seems to be a common phenomenon in intestinal inflammatory disorders. In IBD, an abnormal response to the normal commensal flora of the bowel is considered to cause the disease^[48,49]. The role of the microbiota in the pathogenesis of IBD was

first suggested by studies in mice, which showed a lack of experimental colitis in animals kept in a germ-free environment^[50]. Since then, numerous works have been published in this field. Although no final conclusions can be drawn, it is clear that no single pathogen is associated with the disease, and quantitative besides qualitative changes in the microbiota influence disease development^[51,52].

Microbiota alterations have also been related to CeD risk, again with quantitative and qualitative changes reported^[53,54]. In CeD, an altered microbial diversity depending on the clinical presentation has been recently described, with marked differences between patients showing classical gastrointestinal symptoms and those with extraintestinal manifestations^[55].

The influence of diet (including breastfeeding) and cigarette smoking or the increased risk of disease in children born by Cesarean section have been postulated to be mediated through changes in the microbiota. These changes have also been linked to the increasing incidence of IBD and CeD in recent decades.

Nowadays it is clearly accepted that the intestinal microflora differs between healthy individuals and those showing CeD or IBD. It has been claimed that those differences could be a consequence of the disease. Among other functions, commensal bacteria of the gut contribute to protection against external pathogens and participate in the maturation of the mucosal immune system, supporting their role in the etiology of these diseases. Recent genetic studies are also concordant with a causal role^[56]. Nevertheless, a complex situation exists because the microbiome can be altered as a result of infection or pathological processes.

Genetics

The genetic contribution to disease risk differs between CeD and IBD. The highest values are observed for CeD (75% of concordance between monozygotic twins)^[57], followed by CD (44%-50%) and UC (16%)^[58].

Knowledge of the genetic basis of immune-mediated diseases has dramatically increased in recent years with the advent of genome-wide association studies (GWAS). These studies analyze hundreds of thousands of common [minor allele frequency (MAF) > 5%] genetic variants (single nucleotide polymorphisms, SNPs) across the human genome, looking for variants with a different frequency between individuals showing the disease and the general population. They need high numbers of affected and unaffected individuals that provide enough statistical power to find significant associations. Initially, GWAS included around 1000 individuals with each phenotype, but this number has been increased in recent GWAS. Moreover, follow-up of the nonsignificant most associated SNPs and meta-analysis of previously published largescale studies have also been performed. Additionally, cross-disease meta-analyses that combine data of previous GWAS have been performed to identify susceptibility loci common to different immune-mediated diseases. Looking for variants shared between CD and CeD, these

studies have identified four new shared loci^[59]. New approaches to study the genetic basis of IBD and CeD include the Immunochip Project, also based on the presence of a common genetic basis for immune-mediated diseases but focused on deep replication and fine mapping^[56,60]; and the recently published high-throughput exon-sequencing of 25 GWAS risk genes^[61].

GWAS are not based on prior hypothesis determined by previously available information (*e.g.*, gene function, previous association studies, and animal models) and the studied SNPs are selected to cover a high proportion of gene variation across the genome. With this approach, unexpected genes have been identified as related to disease susceptibility. The newly identified genes point out functional pathways involved in particular phenotypes, some of them also previously unexpected.

IBD and CeD show both a complex genetic basis that is characterized by the presence of numerous common susceptibility factors contributing a small risk to disease susceptibility. In IBD, no factor seems either necessary or sufficient to develop the disease, as it is commonly observed in complex diseases. However, CeD constitutes a particular case. It is commonly accepted that the main genetic risk alleles, those coding the HLA-DQ2 or -DQ8 heterodimers, are necessary although not sufficient to develop CeD, because they are present in almost all CeD patients.

The influence of the HLA region in disease risk marks more differences between IBD and CeD. This region, located on 6p21, contains hundreds of genes with immunological functions and it is responsible for the strongest association signals observed in most immune-mediated diseases. However, HLA influence is different between IBD and CeD, and these two diseases are at opposite ends of the spectrum. HLA loci are the main genetic susceptibility factors for CeD and they are responsible for 40% of the genetic risk; in addition, their functional involvement in disease pathogenesis is well established. On the contrary, a weak and a weak-moderate association is found in CD and UC, respectively. Moreover, the HLA alleles associated with IBD are markers of still unknown HLA risk variants^[62].

Additional to the HLA region, 163 loci have been associated with IBD risk: 110 common to CD and UC, 30 specific for CD and 23 for UC^[56]. For most of the specific loci, the same direction of effect exists in the two forms of IBD and only two loci (*NOD2* and *PTPN22*) have shown significant opposite effects between CD and UC^[56]. In CeD, 40 susceptibility loci have been described^[60,61]. The number of associated SNPs is even higher, because one SNP does not always account for the risk overall attributed to one locus. The causal variant or even the genes responsible for the reported associations remain unknown for many of these regions. In CeD, the individual gene involved is known for half of the associated regions. In both diseases, independent effects of the associated variants have been reported, as well as correlation of genotypes for many SNPs with expres-

sion levels, and an important role of noncoding variants. This last observation has been recently underscored by the discovered negligible impact of rare variants (MAF < 5%) within exons^[61], until now considered as potential relevant contributors to disease risk. The different number of variants associated with IBD and CeD is probably mirroring the different sample sizes used in the studies.

The large sample sizes required by GWAS have been achieved thanks to international collaborations, but this necessary effort may overlook genetic factors associated with specific populations. A new genetic region (22q13.2) has been recently associated with CD risk in a GWAS performed in a Southern European population^[63]. This encourages us to study homogeneous groups of patients (in terms of ethnicity or clinical features) to look for new genetic susceptibility factors.

GWAS have found similar genes associated with IBD when considering childhood or adult onset^[56], although the severe inflammation that mutations in interleukin (*IL*)-10RB cause in children suffering extreme phenotypes, identified through other kind of genetic studies, must be highlighted^[64]. In CeD, studies considering the age of onset remain to be performed.

Seventy percent (113/163) of the IBD loci are shared with other complex diseases, and 12% (20) with CeD. The picture is different when the number of CeD risk regions is used as a reference; 50% of the CeD loci are shared with IBD. Independent of the real percentage, which is impossible to ascertain until scientific advances provide us with a full knowledge of the genetic basis of these diseases, the existence of a common genetic background is evident.

Despite the huge advance in the genetic basis of these chronic diseases, only 14% of the genetic variance is known in CD, 7.5% in UC, and approximately 50% in CeD. The highest values of CeD are due to the strong influence of the HLA, which accounts for 40% of the genetic variance.

IMMUNOPATHOLOGY

The model of immunopathogenesis for CeD has long been established. Dietary gluten induces innate and adaptive immune responses. The innate immune response is characterized by the gluten-induced production of IL-15, which acts on intraepithelial lymphocytes and licenses them to kill epithelial cells. This increases permeability and facilitates that gluten peptides pass through the impaired epithelial barrier into the lamina propria. In this compartment, TG2 induces deamidation of gluten-derived peptides, creating epitopes that bind efficiently to HLA-DQ2/DQ8 heterodimers on antigen-presenting cells, and thus elicits a T-cell response^[65,66].

GWAS findings suggest four main processes underlying CeD: T-cell development in the thymus, innate immune detection of viral RNA, T and B cell co-stimulation (or co-inhibition) and cytokines, chemokines and their receptors. It seems now that a specific enrichment for genes involved in natural killer (NK) cell activation

and interferon γ production also exists. Thus, besides T cells, other cell types may have special relevance in the pathological process, as B cells, NK cells or neutrophils, but the previous model of pathogenesis remains valid and basically unchanged.

In IBD, the model of pathogenesis is based on the dysregulation of the normally controlled immune response to commensal bacteria, which could be precipitated by infection or by defects in the mucosal barrier. This involves infiltration of several cells of the immune system and chemokine and cytokine production, which in turn exacerbate the dysfunctional immune response and activate either T helper (Th)1 or Th2 cells in the gut mucosa, associated with CD and less conclusively with UC, respectively^[67].

GWAS results have been crucial in advancing our understanding of IBD pathogenesis. Two major findings were the unsuspected role of autophagy and the implication of the Th17 immune response. The genes associated with CD and UC risk point to shared pathways involved in the pathogenesis of these two inflammatory conditions.

The genes shared between IBD and CeD are mainly related to the innate immune response against pathogens and to the activation of the immune system to produce inflammation, including T-cell differentiation and immune-cell signaling. Specific pathways like autophagy and Th17 response seem to be only involved in IBD. Autophagy is responsible for degradation of intracellular structures, but it is also important in removal and recognition of invasive pathogens. Its involvement in CD etiology was suggested after the association of *ATG16L1*, *LRRK2* and *IRGM* with CD risk. The implication of Th17 cells marks an important difference between IBD and CeD, because they have been associated with susceptibility to numerous immune-related diseases but not to CeD^[60,68], which is still considered to be a Th1-mediated disease. Th17 cells are involved in defense against extracellular pathogens but they act as potent inducers of autoimmunity through their involvement in tissue inflammation and are probably linked to innate and adaptive responses^[69]. *IL23R* was the first Th17 gene found in genetic association studies, but it was followed by numerous related loci: *IL22*, *IL17A*, *IL17F*, *TYK2*, *JAK2*, *CCR6* and *STAT3*.

In Figure 1, all the genes associated with IBD and CeD in large scale studies are shown. They have been grouped according to their predominant role in three major functions: innate immune response, adaptive immune response, and epithelial barrier function. All the genes with a different role or with still unknown function have been grouped as “others”. The highest number of shared genes between IBD and CeD is observed for genes involved in adaptive immunity. The specific association with UC risk for most of the loci involved in barrier function is noteworthy.

Both CeD and IBD need an environmental stimulus that activates the immune system and leads to the pathological process. The amplification of the immune re-

sponse involves release of cytokines, molecules involved in intracellular signaling, and transcription factors. GWAS have found many genes coding for products related to these processes. The initial stimulus to trigger the disease is different and it seems to be crucial in developing one or other disease, probably in combination with the genes specific for each disease.

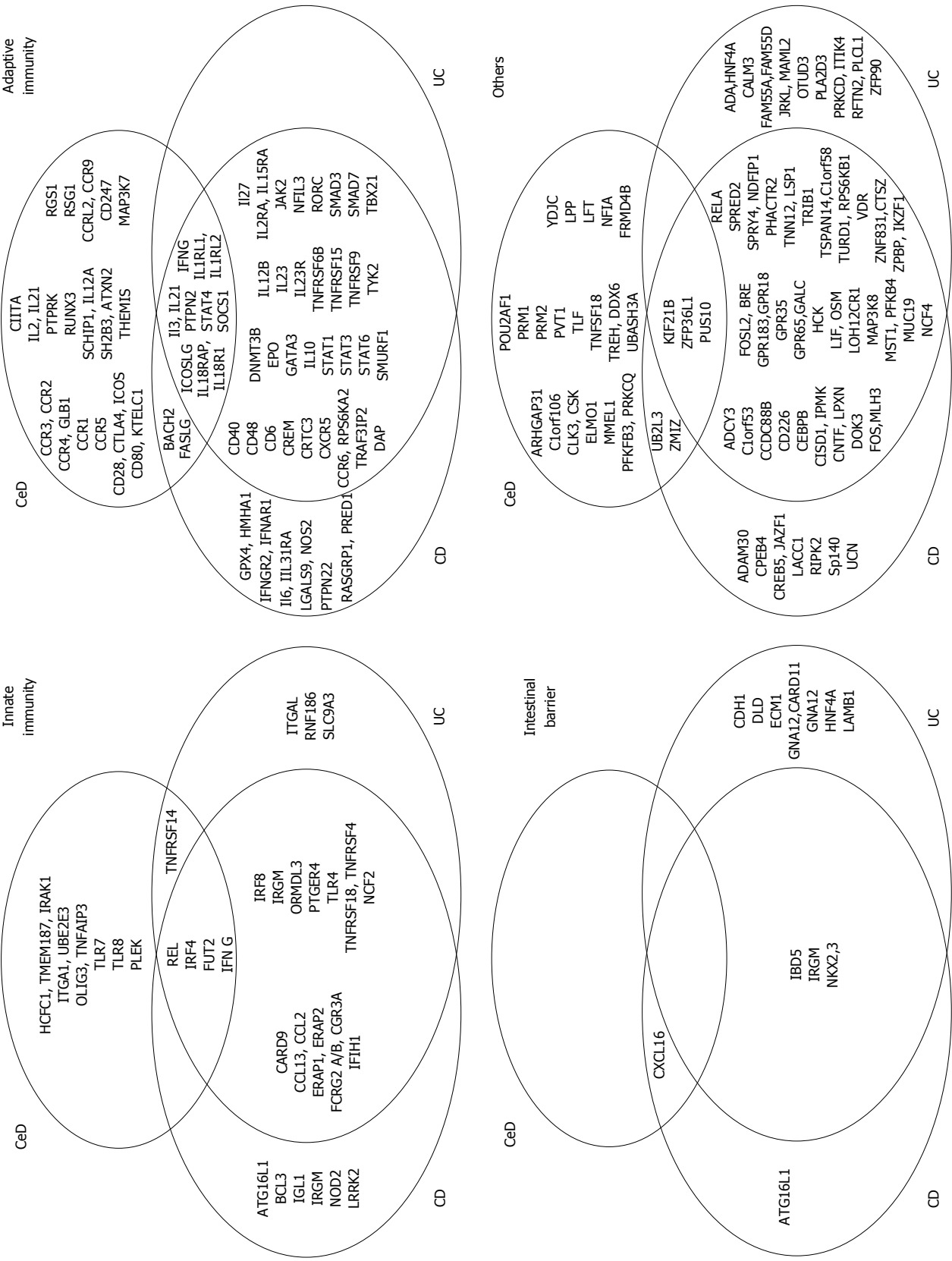
Despite the scientific revolution prompted by GWAS, some issues still hamper a direct translation to disease pathogenesis: (1) in many regions, the genetic variant or even the gene responsible for the association remain to be identified; (2) many of the proteins detected are pleiotropic, with different possible roles and with still unknown functions; and (3) the immune responses, with complex networks of interactions that include specific molecules inducing up- or downregulation of the same functional pathway depending on the microenvironment.

TREATMENT

There is no single effective treatment for all IBD patients. Therefore, different treatments are used to manage the disease, often several of them in the same individual. Conventional immunosuppressive drugs, including azathioprine, mercaptopurine and methotrexate, are the initial treatment for IBD. When the immunosuppressive therapy losses efficacy or the patient continues with active disease, alternative biological therapies based on tumor necrosis factor (TNF)- α blockade are commonly used in IBD. In recent years, infliximab and adalimumab became standardized biological therapies in IBD. Infliximab is a chimeric monoclonal IgG1 anti-TNF α and it is indicated in refractory CD^[70,71] and in acute severe UC^[72]. Despite the high efficacy of infliximab, some patients do not respond to this treatment. An alternative therapy for these patients is adalimumab, an humanized TNF- α antibody, which decreases the risk of developing antibodies^[73-75], one of the causes of non-response to infliximab^[76]. Based on the results of genetic studies, new biological therapies are currently being tested or are already in clinical use in IBD patients^[77-80].

In CeD, treatment is a lifelong GFD. This is an effective and safe treatment, but there is a small group of patients who do not respond. Refractory CeD patients (RCeD) are defined as those showing persistent villous atrophy, crypt hyperplasia, and high levels of intraepithelial lymphocytes despite strict adherence to a GFD for > 12 mo^[81,82]. There are two categories of RCeD, depending on the presence (type I) or not (type II) of aberrant intraepithelial T cells^[83]. RCeD patients develop higher severe malnutrition combined with an increased risk for developing enteropathy-associated T-cell lymphoma^[84]. Immunosuppressive therapy, similar to that used in IBD patients and based on azathioprine, cyclosporine or anti-TNF- α , is the current treatment for RCeD^[84,85]. In the bad prognosis of RCeD, primarily type II RCeD, chemotherapy shows moderate clinical, histological and hematological efficacy^[86,87].

Figure 1 Immunopathology. Overlap between inflammatory bowel disease (IBD) [considering the two major forms Crohn's disease (CD) and ulcerative colitis (UC)] and celiac disease (CeD) for the loci identified by large scale genetic studies. Genes are grouped according to their participation in three main functional blocks: innate immunity, adaptive immunity, and intestinal barrier. All the genes with still unknown function are grouped as "others". Note that some genes can be found in more than one functional group.



New therapeutic approaches in CeD have increased in recent years. These therapies are focused on engineering gluten-free grains, decreasing the intestinal permeability by blockade of the epithelial zonulin receptor, inhibiting gluten peptide presentation by HLA-DQ2 antagonists, and inducing oral tolerance to gluten^[88].

In both disorders, a delay in diagnosis or in proper treatment carries an increased risk of future complications. However, individuals with different autoimmune diseases can present similar symptoms, which sometimes makes it difficult to establish early diagnosis and treatment.

Reconstitution of the physiological flora remains an interesting therapeutic aim for both IBD and CeD.

FUTURE PERSPECTIVES

Despite the great advances in our understanding of IBD and CeD, we are still far from being able to anticipate who will develop some of these immune-mediated conditions. Ingestion of gluten and alterations in the commensal microbiota seem to be the main environmental triggers for CeD and IBD, respectively. However, it remains to be understood what is further needed to break the tolerance in specific individuals and develop disease.

Nevertheless, current advances related to the functional pathways involved in IBD are being useful in finding new drug targets. The shared molecular pathways between IBD and CeD open new possibilities for therapy in RCeD. With the identification of the causal variants in all the associated regions new clues about disease pathogenesis will be obtained and new treatment targets will appear.

Future epidemiological studies are necessary to gain knowledge about the genetic and environmental interactions that contribute to disease development. Better knowledge of the role of pathogens will also be useful to look for new therapies or prevent disease. Although numerous factors make it difficult to study the environmental contributing factors, some genes seem to point to specific triggers. Prospective as well as retrospective studies involving individuals with alterations in those genes could be performed, which aim to advance the role of those specific environmental triggers.

The multifactorial nature of the etiology of CeD and IBD likely hides great complexity due to the interplay among all the factors involved. The role of the microbiota seems to be influenced by interaction with external pathogens, but also by host genetic factors. More research is needed in this field, which will likely contribute to identifying where the missing heritability lies and a better understanding of the immunopathogenesis.

A broad range of symptoms characterize CeD and IBD. Subgroups of patients combining their clinical features with the presence of a similar genetic profile may help to establish more homogeneous groups to perform the next steps in research.

CONCLUSION

IBD and CeD are two immune-mediated disorders with

a partially common genetic background. Overlaps between both disorders are also observed for other specific features; however, these conditions do not seem to be more strongly correlated with each other than with other immune-related disorders. The common clinical manifestations are probably a consequence of the target organ affected: the gut. Shared genetics originates the altered immune response and the inflammation characterizing both diseases. Nevertheless, comparison of these two diseases helps to understand what is specific for each disease and what is common. Common features may be useful to understand better the inflammatory processes and to look for new shared therapies.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Venous thrombosis and prothrombotic factors in inflammatory bowel disease

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increased risk of VTE in IBD: inflammatory activity, hospitalisation, surgery, pregnancy, disease phenotype (*e.g.*, fistulising disease, colonic involvement and extensive involvement) and drug therapy (mainly steroids). There is also convincing evidence from basic science and from clinical and epidemiological studies that IBD is associated with several prothrombotic abnormalities, including initiation of the coagulation system, downregulation of natural anticoagulant mechanisms, impairment of fibrinolysis, increased platelet count and reactivity and dysfunction of the endothelium. Classical genetic alterations are not generally found more often in IBD patients than in non-IBD patients, suggesting that genetics does not explain the greater risk of VTE in these patients. IBD VTE may have clinical specificities, namely an earlier first episode of VTE in life, high recurrence rate, decreased efficacy of some drugs in preventing further episodes and poor prognosis. Clinicians should be aware of these risks, and adequate prophylactic actions should be taken in patients who have disease activity, are hospitalised, are submitted to surgery or are undergoing treatment.

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Key words: Acquired; Genetic; Prothrombotic; Venous thrombosis; Risk of venous thrombosis; Inflammatory bowel disease

Abstract

Patients with inflammatory bowel disease (IBD) may have an increased risk of venous thrombosis (VTE). PubMed, ISI Web of Knowledge and Scopus were searched to identify studies investigating the risk of VTE and the prevalence of acquired and genetic VTE risk factors and prothrombotic abnormalities in IBD. Overall, IBD patients have a two- to fourfold increased risk of VTE compared with healthy controls, with an overall incidence rate of 1%-8%. The majority of studies did not show significant differences in the risk of VTE between Crohn's disease and ulcerative colitis. Several acquired factors are responsible for the

Core tip: In inflammatory bowel disease (IBD), there is an increased risk of venous thrombosis (VTE) due to inflammatory activity, hospitalisation, surgery, pregnancy, disease phenotype and drug therapy. Classical genetic alterations are not generally found more often in IBD patients than in non-IBD patients, suggesting that genetics does not explain the greater risk of VTE in these patients. IBD VTE may have clinical specificities, namely an earlier first episode of VTE in life, high recurrence rate, decreased efficacy of some drugs in preventing further episodes and poor prognosis.

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INTRODUCTION

The possible association between inflammatory bowel disease (IBD) and venous thrombosis (VTE) was first reported in 1936 by Bargen *et al*^[1], who described 18 patients with thromboembolic disease (predominantly venous) from among more than 1000 patients treated for IBD at the Mayo Clinic. Since that time, several publications have suggested that patients with IBD have an increased risk of VTE, including deep venous thrombosis (DVT), pulmonary emboli, portal vein thrombosis, cerebral venous sinus thrombosis, Budd Chiari syndrome and retinal vein thrombosis^[2-5]. The overall incidence rate of VTE in IBD patients has been estimated to be 1%-8%, although necropsy studies report an incidence of 39%-41%^[2-5]. One systematic review^[4] and one meta-analysis^[3] showed a higher VTE risk in IBD patients, even after correction for known prothrombotic factors such as smoking and obesity^[3]. Nevertheless, other studies, such as that of Grip *et al*^[6], show a similar risk between IBD and the background population. However, in that report, where the incidence of VTE in the IBD cohort (0.15% per year) was comparable with that of the background population, the differences in age between the groups could have affected the conclusions^[6].

It is important to stress that most of the evaluated studies were retrospective. When the IBD population was compared with other patients or healthy controls, most of the classical prothrombotic risks were not assessed, and therefore a bias could have been present. Therefore, the aim of this review was to assess the risk of VTE and the prevalence of acquired and genetic VTE risk factors and prothrombotic abnormalities in IBD.

SEARCH STRATEGY

A systematic review was conducted on published articles that assessed the risk of VTE and the prevalence of acquired and genetic VTE risk factors and prothrombotic abnormalities in IBD through a literature search of PubMed, ISI Web of Knowledge and Scopus. This search was performed in September 2013 using the following medical terms: "venous thrombosis IBD", "acquired venous thrombosis risk factor IBD", "genetic venous thrombosis risk factor IBD", "coagulation IBD", "fibrinolysis IBD", "platelets IBD" and "endothelium IBD". Additionally, a comprehensive search of reference lists of all review articles and original papers achieved by this method was performed to identify additional reports that could be included in the final analysis. Potential studies were initially screened by title and abstract. Potential

exclusion criteria to reduce the risk of bias and unnecessary observations included case reports on single patients, book chapters and studies exclusively on arterial thrombosis. A total of 207 articles were studied to construct this review.

RISK OF VTE IN IBD

A summary of the controlled studies comparing the risk of VTE in IBD patients with the risk of VTE in non-IBD patients is presented in Table 1.

General risk

In one of the earliest studies evaluating the incidence of VTE in IBD patients, 61 out of 7199 patients (0.84%) developed VTE during an 11-year period from January 1970 to December 1980 at the Mayo Clinic, with similar rates of VTE observed in patients with Crohn's disease (CD) and ulcerative colitis (UC)^[7]. In 2001, Bernstein *et al*^[8] published the first study on the risk of VTE in IBD in a large population-based study using health administrative data from the province of Manitoba, Canada, in which they applied validated case ascertainment definitions of CD and UC (Table 1). The incidence rate for VTE in IBD patients was 45.6 per 10000 persons-year of follow-up, and IBD patients were 3.5 times more likely to develop VTE than the controls. Similar rates of VTE were observed in CD and UC and in males and females. The highest rates of VTE were observed among patients over 60 years old; however, the highest incidence rate ratio (IRR) for VTE was among patients younger than 40 years old (IRR = 6.02, 95%CI: 3.92-9.12). In 2004, Miehsler *et al*^[9] compared the risk of VTE in patients with IBD and other chronic inflammatory diseases (rheumatoid arthritis and coeliac disease) with matched controls (Table 1). The subjects with IBD had a significantly higher risk of VTE compared with the matched controls [prevalence: 6.15% *vs* 1.62%; odds ratio (OR) = 3.6, 95%CI: 1.7-7.8], whereas the subjects with rheumatoid arthritis or coeliac disease had a risk of VTE similar to that of the controls. In 2007, the risk of VTE among 17 chronic illnesses was evaluated (Table 1)^[10]. The relative risk (RR) of VTE was nearly twofold higher in IBD patients than in the matched controls (OR = 1.84, 95%CI: 1.29-2.63), with only cancer and heart failure carrying a greater risk of VTE than IBD.

IBD, activity, hospitalisation and surgery

Some studies have shown that the risk of VTE may be higher in UC than in CD^[11,12], with other showing the opposite^[13]; however, the majority of did not show significant differences in the risk of VTE between CD and UC^[3,4,8,14,15]. A recent meta-analysis showed similar risks in patients with UC (RR = 2.57, 95%CI: 2.02-3.28; *n* = 6 studies) and CD (RR = 2.12, 95%CI: 1.40-3.20; *n* = 5 studies)^[3].

Several studies reported IBD activity in 45% to 90% of patients at the time of VTE diagnosis^[8,9,16-18]. The asso-

Table 1 Risk of venous thrombosis in inflammatory bowel disease patients relative to non-inflammatory bowel disease patients

Ref.	Design	Population		Risk measure (95%CI)	Controlled variables
		IBD	Controls		
Grip <i>et al</i> ^[6] , 2000 Sweden	Retrospective cohort study Inpatients Records from 2 university hospitals	1253 patients	387 (significant age differences between the IBD cohort and controls)	Incidence rate of VTE 1.5/1000 IBD per year (comparable to the background population)	
Bernstein <i>et al</i> ^[8] , 2001 Canada	Retrospective cohort study Inpatients Manitoba Health administrative 1984-1997	5529 patients	Approximately 55000 year, age, gender and postal area of residence matched members of the general population	DVT RR 4.7 (3.5-6.3) CD RR 2.8 (2.1-3.7) UC PE RR 2.9 (1.8-4.7) CD RR 3.6 (2.5-5.2) UC	
Miehsler <i>et al</i> ^[9] , 2004 Austria	Retrospective cohort study Outpatients and inpatients Three outpatient clinics of Division of Gastroenterology and Hepatology	618 patients	707 age and gender matched controls	Incidence rate of VTE 6.2% IBD 1.6% Controls VTE aOR 3.6 (1.7-7.8) IBD	Operation, injuries, oral contraceptive use, pregnancy, body mass index and smoking
Bernstein <i>et al</i> ^[20] , 2007 Canada	Retrospective cohort study Inpatients The Statistics Canada's Health Person Oriented Information database 1994-2004	About 22000 to 25000 patients	About 2.5 to 3.2 million age and gender matched controls	VTE ≥ 50 yr old RR 1.3 (1.23-1.37) IBD < 50 yr old RR 1.57 (1.42-1.72) IBD	
Huerta <i>et al</i> ^[10] , 2007 United Kingdom	Prospective cohort study with nested case-control analysis Outpatients and inpatients General Practice Research Database - GPRD 1994-2000	6550 patients	10000 age, gender and year matched controls	VTE OR 1.84 (1.29-2.63) IBD	
Nguyen <i>et al</i> ^[12] , 2008 United States	Retrospective cohort study Inpatients Nationwide Inpatient Sample 1998-2004	116842 patients (73197 CD and 43645 UC patients)	522703 controls	VTE aOR 1.48 (1.35-1.62) DC aOR 1.85 (1.70-2.01) UC	Age, gender, calendar year, health insurance payer, comorbidity, presence of IBD related surgery, geographic location, and hospital characteristics
Ha <i>et al</i> ^[148] , 2009 United States	Retrospective cohort study Outpatients and inpatients MarketScan Commercial Claims and Encounters database - Thomson Reuters 2001-2006	17487 patients (7480 CD and 9968 UC patients)	69948 age, gender and index date matched controls	PVT aHR 6.2 ($P < 0.05$) IBD DVT aHR 2.3 ($P < 0.0001$) IBD PE aHR 1.7 ($P < 0.001$) IBD	Hypertension, diabetes, hyperlipidemia, and, in women, the use of contraceptives
Nguyen <i>et al</i> ^[14] , 2009 United States	Retrospective cohort study Pregnant hospitalized women Nationwide Inpatient Sample 2005	3740 patients (2372 CD and 1368 UC patients)	4.21 million pregnant women	VTE aOR 6.12 (2.91-12.9) CD aOR 8.44 (3.71-19.2) UC	Maternal age, race/ethnicity, median neighbourhood income, comorbidity, health insurance, geographical region, hospital location and teaching status and caesarean delivery
Grainge <i>et al</i> ^[19] , 2010 United Kingdom	Retrospective cohort study Outpatients and inpatients General Practice Research Database 1987-2001	13 756 patients (4835 CD and 6765 UC patients)	71672 age, gender, and general practice matched controls	VTE aHR 3.4 (2.7-4.3) IBD	Age, sex, body-mass index, smoking, cancer diagnosis and history of pulmonary embolism or deep vein thrombosis
Novacek <i>et al</i> ^[16] , 2010 Austria	Retrospective cohort study Outpatients IBD patients from 14 Austrian centers specializing in the treatment of patients with IBD (2006-2008) and controls patients from 4 centers in Austria (1992-2008) 2006-2008	86 patients with history of unprovoked VTE	1255 controls with unprovoked VTE	Recurrence 5 yr after discontinuation of anticoagulation therapy aRR 2.5 (1.4-4.2) IBD	Age, gender, factor V Leiden, prothrombin G20210A mutation, high factor VIII (> 234 IU/dL), duration of anticoagulation and body mass index

Scarpa <i>et al</i> ^[147] , 2010 Italy	Prospective case-control study Hospitalized patients who had major colo-rectal surgery Patients admitted for colorectal surgery in the institute of Clinica Chirurgica I of the University of Padova (Italy) 2004-2006	323 patients	432 controls	Incidence rate of VTE in surgical IBD patients <i>vs</i> surgical non IBD patients (both with prophylactic therapy) 1.9% <i>vs</i> 0% VTE with prophylactic therapy OR 5.9 (0.9-39.7) UC All VTE	
Kappelman <i>et al</i> ^[13] , 2011 Denmark	Retrospective cohort study and nested case-control study Danish National Patient Registry 1980-2007	49799 patients (14211 CD and 35 229 UC patients)	477504 age and gender matched members of the general population	HR 2.0 (1.8-2.1) IBD HR 2.2 (2.0-2.5) CD HR 1.9 (1.8-2.0) UC Unprovoked VTE HR 1.6 (1.5-1.8) IBD HR 2.0 (1.6-2.5) CD HR 1.5 (1.4-1.7) UC aOR 1.7 (1.3-2.2) IBD VTE aOR 2.03 (1.52-2.70) IBD	Comorbidities and medications
Merrill <i>et al</i> ^[23] , 2011 United States	Retrospective cohort study Surgical patients National Surgical Quality Improvement Program 2008	2249 patients	269119 patients without IBD who were hospitalized and underwent surgery	aOR 1.7 (1.3-2.2) IBD VTE aOR 2.03 (1.52-2.70) IBD	Age, gender, race/ethnicity, admitted from home, smoker, BMI > 30, medical history, clinical factor
Rothberg <i>et al</i> ^[22] , 2011 United States	Retrospective cohort study Inpatients 374 US hospitals 2004-2005	814 patients	241924 controls	VTE aOR 3.11 (1.59-6.08) IBD	Age, gender, VTE prophylaxis, length of stay ≥ 6 d, primary diagnosis, comorbidities, cancer and treatments
Saleh <i>et al</i> ^[11] , 2011 United States	Retrospective cohort study Inpatients National Hospital Discharge Survey 1979-2005	2932000 patients (1803000 CD and 1129000 UC patients)	918570000 age, gender matched controls	VTE HR 1.08 (1.06-1.09) CD HR 1.64 (1.62-1.66) UC	
Sridhar <i>et al</i> ^[21] , 2011 United States	Cross-sectional study Inpatients Nationwide Inpatient Sample 2010	148229 patients	17261952 controls	VTE (DVT, PE and/or PVT) aOR 1.38 (1.25-1.53) IBD	Hypertension, diabetes mellitus and hyperlipidemia
Bröms <i>et al</i> ^[15] , 2012 Sweden	Retrospective cohort study Pregnant women Medical, Patient, and Prescribed Drug Registers of all residents in Sweden 2006-2009	1996 patients (787 CD and 1209 UC patients) who gave birth to a single infant	10773 women without IBD who gave birth to a single infant	VTE aRR 2.65 (0.65-10.1) CD (with inactive disease) aRR 3.78 (1.52-9.38) UC	Age, parity, smoking, body mass index and comorbidities

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; VTE: Venous thrombosis; DVT: Deep venous thrombosis; PE: Pulmonary emboli; PVT: Portal vein thrombosis; aRR: Adjusted relative risk; aOR: Adjusted odds ratio; aHR: Adjusted hazard ratio.

ciation of VTE and IBD flares was assessed using a large primary care database from the United Kingdom (Table 1)^[19]. According to the data from this assessment, the risk of VTE was increased most prominently during a flare of IBD [hazard risk (HR) = 8.4, 95%CI: 5.5-12.8], compared with periods of chronic activity (HR = 6.5, 95%CI: 4.6-9.2) and periods of clinical remission (HR = 2.1, 95%CI: 1.6-2.9). The RR at the time of a flare, compared with a matched control, was higher during non-hospitalised periods (HR = 15.8, 95%CI: 9.8-25.5 *vs* HR = 3.2, 95%CI: 1.7-6.3). However, this finding must be interpreted with caution because the lower RR during hospitalised periods is related to a higher absolute risk (37.5 *vs* 6.4 per 1000 persons-years), and the treatment with corticosteroids in patients with active disease may also be an additional risk factor for the development of VTE. Moreover, the use of VTE prophylaxis in hospitalised patients can also contribute to a lower RR of VTE during hospitalisation. Bernstein *et al*^[20] showed higher VTE rates in hospitalised IBD

patients than in non-IBD hospitalised patients regardless of age (Table 1). IBD patients who were younger than 50 years had a higher RR than those who were older than 50 years (RR = 1.57, 95%CI: 1.42-1.72 *vs* RR = 1.30, 95%CI: 1.23-1.37)^[20]. Nguyen *et al*^[12] compared the risk of VTE between hospitalised IBD patients and randomly selected hospitalised non-IBD patients (Table 1) and reported that IBD patients had an adjusted 1.7-fold [adjusted OR (aOR) = 1.66, 95%CI: 1.33-2.06] increased rate of VTE compared with non-IBD patients. In 2011, three studies were published showing a 1.1- to 3.1-fold higher risk of VTE in hospitalised IBD patients (Table 1)^[11,21,22].

The risk of VTE in IBD was also evaluated in the surgical setting. Merrill *et al*^[23] compared the risk of VTE between patients with IBD and patients without IBD who underwent surgery in 211 hospitals participating in the American College of Surgeons National Surgical Quality Improvement Program (Table 1). The incidence of VTE was 2.5% in IBD patients (*n* = 57) *vs* 1.0% (*n* = 2608)

Table 2 Prothrombotic risk factors and abnormalities associated with inflammatory bowel disease

Acquired prothrombotic risk factors
Infection or inflammation, previous thromboembolism, age, smoking, malignancy, central venous catheter, surgery, trauma, immobilization, Pregnancy, drugs (oral contraceptives, steroids), antiphospholipid antibody syndrome, hyperhomocysteinemia, fluid depletion
Genetic prothrombotic risk factors
Factor V Leiden, prothrombin mutation, deficiency of protein C, deficiency of protein S, deficiency of antithrombin, PAI-1 mutation, factor XII mutation and MTHFR mutation
Abnormalities of coagulation
↑ TF, factors VII, FXII, FXI, FX and FV, prothrombin and fibrinogen
↓ AT, protein C, protein S, EPCR, TM and TFPI
↑ Prothrombin fragment 1+2, TAT complexes, fibrinopeptide A and fibrinopeptide B
↓ Factor XIII
Abnormalities of fibrinolysis
↓ t-PA
↑ PAI-1 and TAFI
↑ D-dimer
Abnormalities of platelets
↑ Number, activation (CD40L and P-selectin) and aggregation
Abnormalities of endothelium
↓ NO
↑ vWF

PAI-1: Plasminogen activator inhibitor 1; MTHFR: Methylenetetrahydrofolate reductase; TF: Tissue factor; AT: Antithrombin; EPCR: Endothelial cell protein C receptor; TM: Thrombomodulin; TFPI: Tissue factor-pathway inhibitor; TAT: Thrombin-antithrombin; t-PA: Tissue plasminogen activator; TAFI: Thrombin-activatable fibrinolysis inhibitor; CD40L: CD40 ligand; vWF: von Willebrand factor.

in the controls. IBD remained a significant predictor of VTE after multivariate adjustment (OR = 2.03, 95%CI: 1.52-2.70). Another interesting finding of this was the observation that the risk persisted even when procedures on small and large bowels were excluded, with IBD patients undergoing non-intestinal procedures having a 4.45-fold increased risk of VTE compared with non-IBD patients. Furthermore, in a large cohort of surgical IBD patients, bleeding disorders, steroid use, anaesthesia time, emergency surgery, haematocrit < 37%, malnutrition and functional status were identified as potentially modifiable risk factors for postoperative VTE in IBD patients^[24].

Pregnancy

The risk of VTE in IBD was also evaluated during pregnancy and puerperium. According to the Nguyen *et al*^[14] study, based on nationwide inpatient sample data (database containing discharge abstracts from 1054 hospitals in the United States), the aOR of VTE was substantially higher in women with CD (aOR = 6.12, 95%CI: 2.91-12.9) and UC (aOR = 8.44, 95%CI: 3.71-19.2) compared with the non-IBD obstetric population, and this increased risk was independent of whether the women underwent a caesarean section (Table 1). A similar study conducted by Bröms *et al*^[15] in Sweden also showed an increased risk of VTE in pregnant IBD patients compared with non-IBD pregnant patients but showed a lower odds ratio (aOR = 2.65, 95%CI: 0.65-10.1 for CD; aOR = 3.78, 95%CI: 1.52-9.38 for UC) (Table 1). For women with UC, the increased risk of VTE seemed to be highest during pregnancy and not during puerperium like in the general population of women giving birth.

IBD-phenotype risk factors

Several IBD-phenotype risk factors have been shown

to affect the risk of VTE. Nguyen *et al*^[12] reported that fistulising disease was independently associated with a greater VTE risk (OR = 1.39, 95%CI: 1.13-1.70). Colonic involvement in CD patients or extensive disease in UC patients was also associated with an increased VTE risk. A study by Solem *et al*^[17] showed that CD patients with VTE typically had colonic disease involvement (ileocolonic in 56% and colonic in 23%), and most UC patients with VTE (76%) had pancolonic disease. Nguyen *et al*^[12] found a higher risk of VTE in CD patients with colonic-only disease that was 40% higher than the risk of VTE in those with small bowel-only disease. In UC patients, 25% experienced VTE after proctocolectomy, and VTE recurrence rates were not improved by proctocolectomy^[17].

IBD-nonspecific acquired risk factors

In IBD, several nonspecific acquired risk factors, other than those previously discussed, often increase the risk of VTE, such as oral contraceptive/hormone substitution use, smoking and drug therapy (Table 2). In most studies, at least one of the known clinical risk factors for VTE was present in approximately 20%-50% of IBD patients with VTE at the time a TE occurred^[9,16,18,25]. Approximately 30% of these patients may have two or more risk factors^[9].

PROTHROMBOTIC ABNORMALITIES IN IBD

Impact of inflammation on coagulation

Although the causes of the increased risk of VTE in IBD are not yet completely understood, most studies suggest that this risk is largely dependent on the biological and biochemical effects exerted by the activation of the inflammatory pathways (*e.g.*, cells and cytokines) in

the haemostatic system. In fact, there is now convincing evidence from clinical, epidemiological and experimental studies that inflammation and VTE are related^[5,26-28]. Many inflammatory diseases other than IBD, such as systemic lupus erythematosus, Behçet's disease, polyarteritis nodosa and polymyositis/dermatomyositis, have been associated with an increased risk of VTE in several clinical and epidemiological studies^[5,26-28]. This association appears to be the strongest when the time between the exposure and the outcome is short, *e.g.*, when the inflammatory disease was experienced recently or, more specifically, in the active stage of an inflammatory disease (flare-up)^[5,26-28]. In many inflammatory diseases, such as systemic lupus erythematosus and Behçet's disease, VTE may be part of the presentation of those diseases. VTE in IBD may complicate the differential diagnosis with other inflammatory diseases that may also lead to VTE and intestinal inflammation, such as Behçet's disease.

The impact of inflammation on coagulation has been confirmed by several experimental studies showing that inflammatory mechanisms shift the haemostatic balance to favour the activation of coagulation and, in the extremes, VTE^[27].

Tumour necrosis factor- α (TNF- α) and CD40 ligand (CD40L), two inflammatory cytokines, and C-reactive protein (CRP), a liver-synthesised acute phase protein, have been shown to induce the expression of tissue factor (TF) on the cell surface of leucocytes^[29-31]. Interleukin (IL)-6, an inflammatory cytokine, and TNF- α have been shown to lead to thrombin generation^[32,33]. Of the natural anticoagulant pathways, the protein C pathway and heparin-antithrombin pathway have been shown to be downregulated by IL-1 β and TNF- α ^[34,35], whereas the tissue factor pathway inhibitor (TFPI) has been shown to be inhibited by CRP^[36]. There is also evidence that CRP increases the expression of plasminogen activator inhibitor type 1 (PAI-1) and decreases the expression of tissue plasminogen activator (t-PA)^[37,38].

Inflammatory mediators, such as IL-6, increase platelet production. The newly formed platelets appear to be more thrombogenic. For example, the newly formed platelets activate at lower concentrations of thrombin^[39]. Thus, both the platelet count and platelet reactivity are increased in response to inflammatory mediators.

Some authors have proposed the term "endothelial stunning" for the endothelial dysfunction/activation that may be induced by inflammatory cytokines and may thus play a key-role in the association between inflammation and VTE^[40]. For example, CRP has been shown to induce the release of von Willebrand factor (vWF)^[41] and to reduce the production of nitric oxide (NO) by endothelial cells^[42].

As discussed above, in general for inflammatory diseases, there is also convincing evidence from basic science as well as clinical and epidemiological studies that IBD is associated with several prothrombotic abnormalities, including the initiation of the coagulation system, downregulation of natural anticoagulant mechanisms, impairment

of fibrinolysis, increase in the platelet count and reactivity and dysfunction of the endothelium (Table 3)^[43-45].

The mechanisms underlying IBD-associated prothrombotic abnormalities have been the subject of recent experimental and clinical studies. For example, Yoshida *et al.*^[46,47] showed that TNF- α and IL-1 β are both implicated in the enhanced extra-intestinal thrombosis that accompanies experimental colitis [mice with dextran sodium sulphate (DSS)-induced colitis]. The authors noted that exogenous TNF- α and IL-1 β enhanced thrombosis in the arterioles of control mice and that the enhanced thrombus formation in the arterioles of mice with DSS-induced colitis was significantly attenuated in wild-type colitic mice treated with TNF- α or IL-1 β blocking antibodies and in colitic mice deficient for the TNF- α receptor or the IL-1 receptor. The IL-6 concentrations were positively correlated with disease activity and thrombocytosis in patients with UC^[48]. Taken together, these data suggest that inflammatory cytokines such as TNF- α , IL-1 β and IL-6 may play an important role in the inflammation-mediated risk of VTE in IBD.

Abnormalities of haemostasis associated with IBD

Abnormalities of coagulation: Quantitative alterations in key sites of the coagulation cascade that favour clot formation occur in patients with IBD^[44]. These alterations include the elevation of circulating microparticles (including TF-rich microparticles)^[49,50], factor VIIa^[51], factors XIIa and XIa^[52], factors Xa and Va^[52,53], prothrombin^[7,54-56] and fibrinogen^[56,57]. The levels of antithrombin (AT) are significantly lower in the plasma of patients with IBD^[56,58]. Reports on protein C and S deficiency in IBD are conflicting^[48,59-61]. There is also a significantly lower expression of endothelial protein C receptor (EPCR) and thrombomodulin (TM), which impairs protein C activation leading to lower effective protein C activity^[62]. TFPI levels were also shown to be reduced in patients with IBD^[63,64]. Alterations suggestive of the activation of coagulation have also been reported in IBD. These include elevated prothrombin fragment 1+2 (prothrombin F1+2), thrombin-antithrombin (TAT) complexes, fibrinopeptide A (FPA) and B (FPB)^[7,54-56] and decreased factor XIII levels^[57,65].

IBD treatment was also found to influence coagulation abnormalities associated with IBD. For example, the treatment with infliximab induced a significant decrease in the amounts of circulating microparticles in IBD patients^[49].

Abnormalities of fibrinolysis: The circulating concentration of factors involved in the lysis of clots also favours thrombosis in IBD. The plasma levels of t-PA are significantly lower in IBD patients than these levels in the general population^[66,67]. There is also a significant absolute increase in urokinase-type plasminogen activator (u-PA) activity and a decrease in t-PA activity in the inflamed mucosa of IBD patients compared with the control group^[66]. Two proteins that inhibit fibrinolysis,

Table 3 Controlled studies on the prevalence of inherited thrombophilias in inflammatory bowel disease

Ref.	Compared groups	Results							Significance
		Mutation ¹	CD	UC	IBD	IBD-VTE	HC	C-VTE	
Liebman <i>et al</i> ^[101] , 1998 United States	11 IBD-VTE patients and 51 IBD patients without VTE	Factor V Leiden			4%	36%			Significant difference (OR = 14.00, 95%CI: 1.55-169.25)
Over <i>et al</i> ^[107] , 1998 Turkey	63 IBD patients (20 CD and 43 UC patients) and 36 HC	Factor V Leiden	50%	20%			11%		Significant difference for CD <i>vs</i> HC (OR = 6.5, 95%CI: 1.3-18.0)
Haslam <i>et al</i> ^[112] , 1999 United Kingdom	54 IBD patients (30 CD and 24 UC patients) and 55 HC	Factor V Leiden			9.3%		3.6%		Difference not significant
Heliö <i>et al</i> ^[111] , 1999 Finland	563 IBD patients (235 CD and 328 UC patients) and 142 HC	Factor V Leiden Factor XIII mutation	3.4% 5.0%	5.2% 6.1%	4.5% 5.7%		2.1% 3.3%		Differences not significant Differences not significant
Grip <i>et al</i> ^[6] , 2000 Sweden	16 IBD-VTE patients, 99 C-VTE and 288 HC	Factor V Leiden				27%	11%	28%	Significant difference for IBD-VTE <i>vs</i> HC (OR = 3.0, 95%CI: 0.8-11.9)
		Prothrombin mutation				0%	1.8%	7.10%	Differences not significant
Koutroubakis <i>et al</i> ^[61] , 2000 Greece	84 IBD patients (36 CD and 48 UC patients) and 61 HC	Factor V Leiden			8.3%		4.9%		Difference not significant
Papa <i>et al</i> ^[113] , 2000 Italy	52 IBD patients (19 CD and 33 UC patients) and 156 HC	Factor V Leiden Prothrombin mutation			1.9% 1.9%		1.9% 2.6%		Difference not significant Difference not significant
Vecchi <i>et al</i> ^[110] , 2000 Italy	102 IBD (51 CD and 51 UC patients) and 204 HC	Factor V Leiden Prothrombin mutation			1.5% 1.1%		1.2% 0.7%		Difference not significant Difference not significant
		MTHFR mutation			41.1%		47.4%		Difference not significant
Guédon <i>et al</i> ^[102] , 2001 France	15 IBD-VTE, 58 IBD patients without VTE, 110 C- VTE and 84 HC	Factor V Leiden			0%	14.3%	3.6%	15.50%	Significant difference for IBD-VTE <i>vs</i> IBD (<i>P</i> < 0.05)
		Prothrombin mutation			1.7%	14.3%	3.6%	11.80%	Differences not significant
		MTHFR mutation			0%	0%	1.2%	0.90%	Differences not significant
Mózsik <i>et al</i> ^[106] , 2001 Hungary	84 IBD patients (49 CD and 35 UC patients) and 57 HC	Factor V Leiden	14.3%	27.5%			5.3%		Significant difference for CD and UC <i>vs</i> HC (<i>P</i> < 0.05)
Nagy <i>et al</i> ^[108] , 2001 Hungary	78 IBD patients (49 CD and 29 UC patients) and 57 HC	Factor V Leiden	14.3%	27.6%			5.3%		Significant difference for CD and UC <i>vs</i> HC (<i>P</i> < 0.05)
Turri <i>et al</i> ^[103] , 2001 Italy	18 IBD patients with arterial or venous thrombosis, 45 IBD patients without thromboembolic events and 100 HC	Factor V Leiden Prothrombin mutation			2.2% 0%	0% 0%	5% 2%		Differences not significant Differences not significant
Bjerregaard <i>et al</i> ^[120] , 2002 Denmark	106 IBD patients and 4188 HC	Factor V Leiden Prothrombin mutation			5.7%		6.7%		Difference not significant Difference not significant
Magro <i>et al</i> ^[119] , 2003 Portugal	116 IBD patients (74 CD and 42 UC) and 141 healthy controls	Factor V Leiden G20210A Prothrombin mutation	7% 4%	2% 0%			1% 3%		Differences not significant Differences not significant
		MTHFR mutation	14%	12%			10%		Differences not significant
Saibeni <i>et al</i> ^[121] , 2003 Italy	152 IBD patients (62 CD and 90 UC patients) and 130 HC	PAI-1 mutation Factor XIII mutation	11% 14%		5.3%		24% 5.4%		Differences not significant Difference not significant
Törüner <i>et al</i> ^[118] , 2004 Turkey	62 IBD patients (28 CD and 32 UC patients) and 80 HC	Factor V Leiden Prothrombin mutation			3.2% 0%		6.3% 2.5%		Difference not significant Difference not significant
		MTHFR mutation			11.3%		6.3%		Difference not significant
Mahmood <i>et al</i> ^[117] , 2005 United Kingdom	68 IBD patients (31 CD and 37 UC patients) and 30 HC	Factor V Leiden Prothrombin mutation	0% 0%	1.5% 1.5%	1.5% 1.5%		0% 0%		Differences not significant Differences not significant

Oldenburg <i>et al</i> ^[98] , 2005	22 IBD-VTE patients and 23 IBD patients without VTE	Factor V Leiden	0%	20%				Difference not significant
Netherlands		Prothrombin mutation	8.7%	4.5%				Difference not significant
Spina <i>et al</i> ^[105] , 2005	47 IBD-VTE patients and 94 C-VTE	Factor V Leiden		2.1%	13.8%			Significant difference for C-VTE vs IBD-VTE ($P < 0.05$)
Italy		Prothrombin mutation		8.5%	12.8%			Difference not significant
Yilmaz <i>et al</i> ^[116] , 2006	27 IBD patients and 27 HC	Factor V Leiden	6.7%	5%				Difference not significant
Turkey		Prothrombin mutation	3.3%	6.7%				Difference not significant
		Factor XIII mutation	5%	0%				Difference not significant
		MTHFR mutation	3.3%	0%				Difference not significant
		PAI-1 mutation	11.7%	8.3%				Difference not significant
Bernstein <i>et al</i> ^[109] , 2007	492 IBD patients (327 CD and 165 UC) and 412 HC	Factor V Leiden	6.4%	4.2%	6.1%			Differences not significant
Canada		Prothrombin mutation	1.8%	1.2%	1.2%			Differences not significant
		Factor XIII mutation	8.7%	7.1%	4.3%			Significant difference for CD vs HC ($P < 0.05$)
		MTHFR mutation	12.5%	9.9%	11.7%			Differences not significant
Koutroubakis <i>et al</i> ^[104] , 2007	30 IBD patients with vascular complications, 60 IBD patients without vascular complications, 30 controls with vascular complications and 54 HC	Factor V Leiden	6.7%	20.0%	3.7%	16.7%		Significant difference for IBD-VTE vs HC ($P < 0.05$)
Greece		Prothrombin mutation	5.0%	10.0%	1.9%	13.3%		Differences not significant
		Factor XIII mutation	3.3%	0%	1.90%	0%		Differences not significant
		MTHFR mutation	11.6%	6.6%	7.5%	13.3%		Differences not significant
		PAI-1 mutation	20%		13.0%			Significant difference for IBD-VTE vs HC ($P < 0.05$)
Yasa <i>et al</i> ^[115] , 2007	27 IBD patients and 47 HC	Factor V Leiden	11.1%	43.3%	4.3%	36.7%		Difference not significant
Turkey		Prothrombin mutation	7.4%		0%			Difference not significant
		MTHFR mutation	14.9%		6.3%			Difference not significant
Maher <i>et al</i> ^[114] , 2010	26 IBD patients (7 CD and 19 UC patients) and 40 HC	Factor V Leiden	3.8%		2.5%			Difference not significant
Saudi Arabia								
Novacek <i>et al</i> ^[116] , 2010	102 IBD patients (77 CD and 25 UC) and 102 HC	Factor V Leiden	16.1%		26.1%			Difference not significant
Austria		Prothrombin mutation	1.7%		6%			Difference not significant

VTE: Venous thrombosis; CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; IBD-VTE: Inflammatory bowel disease with venous thrombosis; HC: Healthy controls; C-VTE: Controls with venous thrombosis; OR: Odds ratio. ¹Presented prevalence data refer to heterozygous and homozygous carrier for FV Leiden and prothrombin mutation and to homozygous carrier for the other mutations.

PAI-1^[66] and thrombin-activatable fibrinolysis inhibitor (TAFI)^[68], were found in higher concentrations in the plasma of IBD patients. Increasing levels of D-dimer, a fibrin degradation product, were also found in IBD, mainly in patients with active disease^[58]. As D-dimer is generated from cross-linked fibrin, but not from fibrinogen, and elevated plasma concentration of D-dimer indicates recent or ongoing intravascular blood coagulation.

Abnormalities of platelets: In patients with IBD, there are often increased circulating platelet numbers and platelet and leukocyte-platelet aggregates (PLAs)^[69]. Greater platelet aggregation has also been demonstrated in the mesenteric vasculature compared with non-IBD controls, supporting the hypothesis that platelet activity is stimulated in the mesenteric microcirculation^[70]. Indeed, spon-

taneous platelet aggregation or platelet hypersensitivity to low levels of aggregating agents occurs in nearly one-half of patients with IBD^[71] and appears to be independent of the disease activity^[57,71]. Such platelet hyperactivation is mediated at least partly by the CD40-CD40L pathway, a key regulator and amplifier of immune-inflammatory reactivity and inducer of TF, which initiates the extrinsic coagulation pathway. Evidence for the involvement of CD40L includes a markedly elevated expression of CD40L protein by platelets from patients with IBD and the release of larger amounts of soluble CD40L into the plasma, leading to an approximately 15-fold increase in the CD40L plasma levels^[72,73]. There are also elevated levels of CD40L in the mucosa that appear to be proportional to the degree of inflammation^[72,74]. The activation of platelets in IBD may also be mediated by P-selectin.

IBD patients have been reported to have more platelets expressing P-selectin (marker of platelet activation) than healthy controls^[69]. In the DSS model of murine colonic inflammation, colonic inflammation has been reported to be associated with an increased number of circulating activated platelets, along with the formation of PLAs, which can be inhibited by selectin blockade with fucoidin^[75].

The IBD treatment may influence platelet abnormalities associated with IBD. Infliximab significantly reduced plasma-soluble CD40L levels and eliminated CD40 from mucosal microvessels^[76], whereas IBD patients on thiopurines had fewer PLAs than those not taking them^[69]. These findings suggest that IBD treatment may influence platelet abnormalities associated with IBD.

Abnormalities of endothelium: Endothelial dysfunction has been clearly demonstrated in IBD patients and involves several aspects of endothelium biochemical physiology^[77]. In particular, such dysfunction involves an alteration in the NO and reactive oxygen species (ROS) balance, which occurs when the endothelium fails to generate NO, a potent vasodilator and anti-aggregating agent, and instead forms elevated levels of superoxide anion^[78]. Decreased NO generation may result from an acquired deficient transcription of nitric oxide synthase 2 (NOS2) in chronically inflamed IBD endothelium^[79] and from the induction, by many inflammatory cytokines (*e.g.*, IL-2 and TNF- α), of the enzyme arginase, which competes with NOS^[80]. The increased production of ROS in the inflamed endothelium may also contribute to oxidative stress in vWF molecules, which become unresponsive to proteolysis by ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), leading to the accumulation of ultra-large vWF multimers^[81]. The latter are the most haemostatically active forms of vWF and, by favouring platelet adhesion and aggregation, may contribute to microvascular thrombosis in IBD. Increased levels of vWF were reported in IBD patients, especially in those with active disease^[58,82].

Thrombophilia and IBD

Acquired risk factors: Antiphospholipid antibodies (APLA) and hyperhomocysteinaemia are two acquired thrombophilias associated with arterial and venous thrombosis. APLA are a group of prothrombotic antibodies directed against plasma proteins that are bound to anionic phospholipids. Patients with APLA may present with venous or arterial thrombosis, recurrent foetal loss and/or thrombocytopenia. The disorder may be primary or may be associated with pregnancy or with inflammatory, post infectious and other disease states. This group of antibodies includes anti-cardiolipin (aCL), anti- β 2-glycoprotein 1 (b2-GPI) and lupus anticoagulants, each of which requires specific testing. Available studies in IBD vary in the assessment of different antibodies, types of IBD and disease activity level; therefore, the true prevalence of APLA in IBD patients remains unclear. The prevalence of aCL antibodies in IBD patients is higher

than in the control population, with an average incidence of 20%-30%, but the association with thrombosis in IBD patients is less clear^[83]. Similarly, the levels of antibodies against b2-GPI, the cofactor that mediates the binding of aCL antibodies to cardiolipin and a more specific measure of the risk associated with thrombosis, has been detected in 9% of patients with IBD compared with its absence in healthy controls^[84]. Lupus anticoagulants were not detected in a small series of 16 patients with CD^[85]. The levels of aCL antibodies and anti-b2-GPI antibodies and of lupus anticoagulants were similar in a population of IBD patients with and without current or past VTE events^[84,86]. One of the causes of the appearance of APLA in IBD may be anti-TNF- α therapy because this therapy has been associated with the development of APLA^[87,88].

Hyperhomocysteinaemia may be both a genetic and acquired abnormality. The most common genetic defect is homozygosity for a thermolabile mutant of the enzyme methylenetetrahydrofolate reductase (MTHFR)^[89]. Plasma homocysteine concentrations can also be increased by deficiencies in vitamin B6, B12 or folic acid (dietary, genetic or drug-associated)^[90]. Hyperhomocysteinaemia is an independent risk factor for atherosclerotic vascular disease, with the risk increasing in a graded fashion with increasing plasma homocysteine concentrations^[91,92]. Hyperhomocysteinaemia has also been associated with an increased risk of VTE^[93,94]. Reducing levels of homocysteine with B vitamin supplements, however, has not resulted in a reduction in the incidence of recurrent VTE or arterial thrombotic complications^[95,96]. The association of folate deficiency and hyperhomocysteinaemia has been evaluated in IBD patients. One study reported elevated serum homocysteine and low folate in 63 patients with IBD, but these levels were not observed in 183 matched controls without thrombotic complications^[97]. In a small study, fasting homocysteine levels in IBD patients with a history of arterial or venous thrombosis tended to be higher (although not significantly) than in IBD controls^[98]. Recently, Oussalah *et al*^[99] conducted a systematic review and meta-analysis to evaluate the association between homocysteine metabolism and IBD and the association between hyperhomocysteinaemia and thrombosis in IBD. The mean plasma homocysteine level was significantly higher in IBD patients compared with the controls. The mean plasma homocysteine level did not differ between patients with UC and CD. The risk of hyperhomocysteinaemia was significantly higher in IBD patients compared with controls (OR = 4.65, 95%CI: 3.04-7.09). The risk of hyperhomocysteinaemia was not higher among IBD patients who experienced thromboembolic complications (OR = 1.97, 95%CI: 0.83-4.67), suggesting that hyperhomocysteinaemia may not be a major contributor to VTE in IBD. Medication-associated folate deficiency (*e.g.*, methotrexate or sulfasalazine) may be the most common explanation for hyperhomocysteinaemia in IBD patients, although deficiencies in vitamin B6 and B12 and a MTHFR mutation may also play a role

(see below). Taken together, the current data do not support a major role for APLA or hyperhomocysteinaemia in IBD-associated VTE, but studies with a higher number of patients and a prospective design are needed.

Inherited risk factors: A summary of controlled studies on the prevalence of inherited thrombophilias in IBD is presented in Table 3. The rate of inherited thrombophilias in patients with IBD and VTE is estimated to be 15%-30%, which is similar to the rate in non-IBD patients and VTE in most studies^[6,17,25,98,100-104], although one study has reported a lower rate of inherited thrombophilias in patients with IBD and VTE^[105]. Data comparing the prevalence of inherited thrombophilias in the overall IBD population and in the general population are conflicting. Although some cohorts reported a higher prevalence in the overall IBD population^[106-109], most reported a similar prevalence^[6,16,61,102-104,109-121]. These data suggest that the role of inherited thrombophilias in VTE in IBD patients is similar to that in the general population.

The most prevalent thrombophilia reported in IBD patients is factor V mutation. Other genetic variants that have been found in IBD patients include the prothrombin G20210A mutation, deficiencies in protein C, protein S and antithrombin, the PAI-1 4G mutation, the factor XII val34leu mutation and the MTHFR C677T mutation (Tables 2 and 3). Factor V mutation increases the risk of thrombosis five- to eightfold for heterozygous carriers and 50- to 80-fold for homozygous carriers^[122]. A similar frequency of factor V Leiden was reported in IBD patients with thrombosis compared with thrombotic controls^[6,102,104] and in the overall IBD population compared with the general population^[61,109]. However, the prevalence of factor V mutation in thrombotic IBD patients has been shown to be significantly higher than that in IBD patients without thrombosis, suggesting that factor V Leiden, when present, increases the risk of IBD-associated VTE^[101,102]. Two recent meta-analyses confirmed this conclusion. In the meta-analysis of Zhong *et al.*^[123], the OR of VTE in IBD patients with factor V mutation was higher than in IBD patients (OR = 4.00, 95%CI: 2.04-7.87) and healthy controls (OR = 3.19, 95%CI: 1.38-7.36). Liang *et al.*^[124] showed a similar prevalence of factor V mutation in IBD patients and the general population (summary OR = 1.13, 95%CI: 0.87-1.46). Of note, the factor V Leiden mutation was associated with a significantly higher risk of thromboembolism in IBD patients (summary OR = 5.30, 95%CI: 2.25-12.48)^[124].

The prothrombin G20210A mutation leads to greater prothrombin plasma levels (heterozygous carriers have approximately 30% higher PT levels than healthy controls) and increases the risk of VTE approximately threefold^[122]. There is no difference in the prevalence of the prothrombin G20210A mutation between IBD patients and normal controls^[110,120], between IBD patients with thrombosis and non-IBD patients with thrombosis^[6,102] or between IBD patients with and without thrombosis^[102,104]. Protein C, protein S and antithrombin III deficiencies also

have no increased prevalence among patients with IBD, regardless of whether they have had a VTE^[52,125-127].

The factor XIII val34leu mutation is associated with a greater FXIII activation rate and leads to a 20%-40% reduction of the risk of VTE for homozygous carriers^[111,121]. A slightly greater prevalence of factor XIII (val34leu) mutation carriers in CD was found in a recent population-based study^[109], but this prevalence could not explain the greater risk of VTE in CD. Available data suggest that there is no difference in the prevalence of homozygous carriers of the factor XIII val34leu mutation between IBD patients and healthy controls^[111,116,121]. Finally, the prevalence of the factor XIII val34leu mutation was similar in thrombotic IBD patients and non-IBD thrombotic patients^[104].

The MTHFR C677T mutation leads to a 25% increase in homocysteine plasma levels in homozygous carriers^[128]. The effect of the MTHFR C677T mutation on the risk of VTE varies among studies, and a recent meta-analysis found a weak effect (20% risk increase)^[128]. The prevalence of the MTHFR C677T mutation in IBD has shown discordant results, most likely because of regional and ethnic variations in the prevalence of this polymorphism in the general population. The allelic frequency of MTHFR C677T has been reported to be higher in IBD patients than in the reference population^[119]. In a recent population-based case-control study, some differences were observed between patients with IBD and healthy controls (with a decreased number of mutant allele carriers in UC); however, these differences did not explain the excess risk of thrombosis^[109]. No difference in the prevalence of homozygous carriers of the MTHFR C677T mutation was found between the IBD patients and healthy controls in most studies^[102,104,109,110,115,116,118,119]. The prevalence of C677T homozygosity between IBD thrombotic patients and non-IBD thrombotic patients showed no significant difference^[102,104].

Several studies have demonstrated that the PAI-1 (4G) homozygosity is associated with enhanced PAI-1 expression^[129] and contributes as an additional risk factor towards the development of VTE^[130]. However, the evidence regarding the relationship between an elevated PAI-1 plasma level or PAI-1 4G polymorphism and the risk of VTE is rather conflicting. The allelic frequency of PAI-1 4G has been reported as being higher in IBD patients than in the reference population^[119]. Moreover, a recent study showed a significantly higher allelic frequency of PAI-1 4G in IBD patients with vascular complications compared with IBD patients and healthy controls^[104]. No difference in the prevalence of homozygous carriers of the PAI-1 4G mutation was found between IBD patients and healthy controls in most studies^[104,116,119]. The prevalence of this genotype does not differ in thrombotic IBD patients compared with non-IBD thrombotic patients^[104].

As in the general population, more than one thrombotic defect can occur among IBD patients with inherited thrombophilia, particularly factor V Leiden^[104,119]. A higher prevalence of the carriage of two or more throm-

bogenic polymorphisms has been found in IBD patients compared with the reference population^[119], but no significant difference has been found between thrombotic and non-thrombotic IBD patients^[104].

Taken together, these data show that genetic risk factors are generally not found more often in IBD patients than in others, suggesting that genetics does not explain the greater risk of VTE in CD and UC. However, when genetic risk factors occur, patients with IBD (compared with healthy controls) are more likely to suffer thromboembolic complications, suggesting that hereditary thrombophilia and inflammation-associated thrombogenicity have at least an additive effect for the risk of VTE in IBD.

PHARMACOLOGICAL EFFECT ON RISK FACTORS

Almost all drugs used in the treatment of IBD have been associated with abnormalities in the haemostatic system in experimental and clinical studies. Corticosteroids have been associated with both hypo- and hypercoagulating alterations^[131,132]. A meta-analysis demonstrated that dexamethasone-based chemotherapy was a risk for VTE in patients with multiple myeloma^[133]. Furthermore, in a large cohort of surgical IBD patients, the use of steroids was identified as a potentially modifiable risk factor for postoperative VTE in IBD patients^[24]. Studies of platelets from IBD patients treated with 5-aminosalicylic acid (5-ASA) agents have shown conflicting results. *In vitro*, 5-ASA significantly reduced both spontaneous and thrombin-induced platelet activation^[134]. *In vivo*, platelets from IBD patients taking 5-ASA have decreased expression levels of P-selectin, a surface marker for platelet activation^[134], and lower plasma levels of RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted), a prothrombotic platelet cytokine^[135]. In contrast to these findings, a study of six patients with IBD (four with UC and two with CD) treated with 5-ASA showed no changes in platelet aggregation or fibrinolytic activity^[136]. Sulfasalazine inhibits dihydrofolate reductase leading to folate deficiency, which is a cause of hyperhomocysteinaemia (acquired thrombophilia; see above). Plasma homocysteinemia levels have been reported to be significantly increased in patients with ankylosing spondylitis under sulfasalazine therapy^[137].

Azathioprine has been shown to inhibit platelet aggregation *in vitro*^[138]. IBD patients taking thiopurines experienced fewer PLAs than patients who were not taking them^[69]. The *in vitro* data suggest an antithrombotic effect from azathioprine and 6-mercaptopurine. Methotrexate, a folate antagonist, is a well-established contributor to hyperhomocysteinaemia (which is associated with thrombosis risk) when used in patients with rheumatoid arthritis^[139]. Nonetheless, no study associating methotrexate with hyperhomocysteinaemia is available for IBD. Cyclosporine has been associated with thrombogenicity *in vitro* and *in vivo*. *In vitro* studies showed an increased plate-

let aggregation^[138] and activation of endothelial cells^[140] induced by cyclosporine. Cyclosporine has also been associated with impaired fibrinolysis through a decrease in PAI-1 activity^[141]. The *in vitro* thrombogenicity of cyclosporine has been confirmed *in vivo* by several studies showing thrombotic events in patients taking cyclosporine^[142].

Infliximab, an antibody against anti-TNF- α , may decrease platelet activity through the downregulation of the CD40/CD40L pathway in the mucosal microcirculation^[76]. Additionally, in patients with active rheumatoid arthritis, infliximab treatment has been shown to normalise the disease-associated impairment of the coagulation and fibrinolytic systems by decreasing the levels of prothrombin F1+2 and D-dimer^[143], t-PA antigen, PAI-1 antigen and PAI-1 activity^[144]. Finally, infliximab induced a significant decrease in the amounts of circulating microparticles in IBD patients^[49]. Despite these potential anticoagulant effects of the TNF- α blockade, there are also case reports of thrombosis at several sites, such as the retinal vein, in patients under anti-TNF- α therapy^[145]. Moreover, the prothrombotic effects of anti-TNF- α therapy may be mediated by antiphospholipid antibodies (acquired thrombophilia) as anti-TNF- α therapy has been associated with the development of APLA^[87,88]. Nonetheless, a recent prospective observational cohort study of biological safety in patients with rheumatoid arthritis showed that VTE events are not increased in patients with rheumatoid arthritis who are treated with anti-TNF therapy^[146].

CONCLUSION

In IBD, there is an increased risk of thromboembolic events due to inflammation, nutritional deficiencies, hospitalisations, surgery and inherited prothrombotic factors. Moreover, beyond an increased risk, VTE may have clinical specificities in IBD. There is evidence that subjects with IBD experience the first episode of VTE early in life^[6]. In IBD, the RR of VTE is inversely correlated with age (*i.e.*, younger IBD patients have a higher RR of VTE); nevertheless, the actual incidence increases with age^[8,11,13,21]. The rate of recurrent VTE in the five years after the discontinuation of anticoagulation therapy is also increased in IBD [adjusted RR (aRR) = 2.5, 95%CI: 1.4-4.2]^[16]. Even with continued prophylaxis for VTE, the risk of recurrence of VTE in IBD patients has been reported to be 13%^[17]. Low molecular weight heparin (LMWH) has been shown to be less effective in preventing DVT in hospitalised subjects undergoing surgery for IBD than in patients with non-IBD conditions, including colorectal cancer and diverticular disease (aOR = 5.9, 95%CI: 0.9-39.7, for UC and DVT postoperatively)^[147]. Importantly, VTE appears to carry a poorer prognostic outcome for patients with IBD than for the general population. In a hospitalised cohort, the rate of VTE was not only higher in IBD subjects than in the controls, but the admissions for IBD subjects were also longer (11.7 *vs*

6.1 d, $P < 0.0001$) and were associated with higher costs (\$47515 *vs* \$21499, $P < 0.0001$) and higher mortality (aOR = 2.5, 95%CI: 1.83-3.43)^[12].

Therefore, clinicians should be aware of these risks so that adequate prophylactic actions can be taken in all IBD patients with flares, particularly in patients who are hospitalised, submitted to surgery or undergoing treatment.

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New serological markers in pediatric patients with inflammatory bowel disease

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and a significant number of patients undergo surgery during the disease course. Based on recent knowledge, serum antibodies are qualitatively and quantitatively associated with complicated CD behavior and CD-related surgery. Pediatric UC is characterized by extensive colitis and a high rate of colectomy. In patients with UC, high levels of anti-CBir1 and pANCA are associated with the development of pouchitis after ileal pouch-anal anastomosis. Thus, serologic markers for IBD can be applied to stratify IBD patients into more homogeneous subgroups with respect to disease progression. In conclusion, identification of patients at an increased risk of rapid disease progression is of great interest, as the application of early and more aggressive pharmaceutical intervention could have the potential to alter the natural history of IBD, and reduce complications and hospitalizations.

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Pediatric; Serologic markers; Antimicrobial antibodies; Anti-glycan antibodies; Pancreatic antibodies; Inflammatory bowel disease

Abstract

The spectrum of serological markers associated with inflammatory bowel disease (IBD) is rapidly growing. Due to frequently delayed or missed diagnoses, the application of non-invasive diagnostic tests for IBD, as well as differentiation between ulcerative colitis (UC) and Crohn's disease (CD), would be useful in the pediatric population. In addition, the combination of pancreatic autoantibodies and antibodies against *Saccharomyces cerevisiae* antibodies/perinuclear cytoplasmic antibody (pANCA) improved the sensitivity of serological markers in pediatric patients with CD and UC. Some studies suggested that age-associated differences in the patterns of antibodies may be present, particularly in the youngest children. In CD, most patients develop stricturing or perforating complications,

Core tip: Application of non-invasive diagnostic tests for the diagnosis of inflammatory bowel disease (IBD) and differentiation between ulcerative colitis (UC) and Crohn's disease (CD) would be useful in the pediatric population. The combination of pancreatic autoantibodies and antibodies against *Saccharomyces cerevisiae* antibodies/perinuclear cytoplasmic antibody improved the sensitivity of serological markers in pediatric patients with CD and UC. In addition, serologic markers for IBD can be applied to stratify IBD patients into more homogeneous subgroups with respect to disease progression. With this knowledge, clinicians will be able to stratify patients accordingly with regards to the risk of disease progression, create a personalized treatment strategy, and attempt to modify disease course, thereby

improving long-term prognosis.

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INTRODUCTION

Inflammatory bowel diseases (IBD), Crohn's disease (CD), and ulcerative colitis (UC) are chronic relapsing and remitting disorders of the digestive tract with unknown etiology^[1]. Previous studies suggested that IBD results from an aberrant innate and acquired immune response to commensal microorganisms in genetically susceptible individuals^[2,3]. This hypothesis is supported by the presence of antibodies directed to microbial antigens and by the identification of genetic polymorphisms, such as *NOD2/CARD15* and toll-like receptor 4 variants in CD^[4]. Besides genetic predisposition and environmental factors, innate immunity is assumed to be another major contributor to pathogenesis in IBD.

Incidence of IBD is increasing, especially in pediatric patients with CD^[5]. It is estimated that 15%-25% of IBD patients present in childhood. Recent studies showed that up to 20% of pediatric patients and 5%-15% of adult patients with colon only involvement had diagnostic difficulties if they had UC or colonic CD^[6]. Serologic markers may help to establish diagnosis of IBD and to differentiate CD from UC, particularly when they are combined. It is especially important in the pediatric population, where invasive diagnostic testing is less desirable. In CD, most patients develop stricturing or perforating complications, and a significant number of patients undergo surgery during the disease course. Pediatric UC is more often associated with pancolitis and colectomy. Besides their diagnostic significance, current knowledge suggests that serologic markers can be a valuable aid in stratifying patients according to disease phenotype and risk of complications in IBD.

Several circulating autoantibodies have been described in IBD. The two most intensively studied conservative antibodies are atypical perinuclear anti-neutrophil cytoplasmic antibodies (atypical pANCA), which are primarily associated with UC and anti-*Saccharomyces cerevisiae* antibodies (ASCA), which are primarily associated with CD^[4,7]. In pediatric IBD, sensitivity/specificity of pANCA in UC ranged between 57% to 83% and 65% to 97%, respectively, whereas in CD, ASCA showed a sensitivity/specificity in the range of 44% to 76% and 88% to 95%, respectively^[8,9]. ASCA positivity or high titers are associated with complicated CD behavior (penetrating or stenosing disease) and could be useful markers for predicting the need for surgery in adults and children^[10-12].

In pediatric studies, ASCA positivity increased with age at diagnosis^[13] and was predictive for a more relapsing disease course [OR 2.9 (95%CI: 1.33-6.33)] in CD^[14]. In addition, Trauernicht and Steiner^[15] reported that serum ASCA antibodies are associated with lower anthropometric data (lower mean weight and height Z-scores) at the diagnosis of pediatric CD. pANCA is noted for its association with the "UC-like" phenotype in patients with CD^[16,17]. Testing for ASCA and pANCA alone may have limited usefulness; therefore additional seromarkers are needed to improve the diagnosis, differentiation, and stratification of IBD, as well as prediction of disease course.

NEW SEROLOGICAL MARKERS

Crohn's disease

Antibodies to *Escherichia coli* outer membrane porin C, *Pseudomonas*-associated sequence I2, and bacterial flagellin CBir: Several antibodies against microbial components have been detected in serum samples of patients with IBD, including ones against outer membrane porin C (anti-OmpC) of *Escherichia coli*, against *Pseudomonas*-associated sequence I2 (anti-I2), and against bacterial flagellin CBir (anti-CBir1). Adherent-invasive *E. coli* has been found in ileal CD lesions, and OmpC has been shown to be required for these organisms to adhere to intestinal epithelial cells^[18,19]. I2 was identified as a bacterial sequence from lamina propria mononuclear cells of active CD patients, and was shown to be associated with *Pseudomonas fluorescens*^[20]. CBir1 is a flagellin related antigen that was initially identified in the gut flora of mice, and has the ability to induce colitis in immunodeficient mice^[21].

Approximately 50% of adult patients with CD were positive for these markers, which were insignificant in adult patients with UC and healthy subjects^[22,23]. The prevalence of anti-OmpC and anti-I2 was found to be 11% and 56% in pediatric CD, respectively^[10,13,24-28]. The occurrence of antibodies varies in children of different ages: children younger than 8 years old at diagnosis are predominantly anti-CBir1 positive and ASCA and anti-OmpC negative, while those older than 8 are more commonly both ASCA and anti-CBir1 positive^[13]. In children with CD, these strong serological responses to bacterial flagellin CBir antigens suggest that this antigen may have a potential role in the immunopathology of the disease.

Anti-glycan antibodies: The most recently described serum markers directed against microbial antigens are anti-glycan antibodies. Glycans are predominant cell surface oligosaccharides found on microorganisms, immune cells, erythrocytes, and tissue matrices. In IBD, the presence of anti-glycan antibodies results from the interaction between the immune system and the glycosylated cell wall components of such pathogens as fungi, yeast, and bacteria. Besides gASCA (which is very similar to conventional ASCA IgG), certain novel anti-glycan

antibodies were identified and associated with CD: anti-mannobioside carbohydrate antibodies (AMCA), anti-laminaribioside carbohydrate antibodies (ALCA), anti-chitobioside carbohydrate antibodies (ACCA), anti-laminarin carbohydrate antibodies (anti-L), and anti-chitin (anti-C) carbohydrate antibodies.

Anti-glycan markers are significantly increased in CD compared to UC and healthy controls^[29,30]. However, only 16.9%-30.5% of patients were positive for each of AMCA, ALCA, ACCA, anti-L, and anti-C markers in pediatric CD^[31]. Since the presence of anti-L and anti-C is low in ASCA-negative patients with CD, it has been proposed that these markers may bind different epitopes. Interestingly, the optimal cutoff values for anti-glycan markers were different in children than in adult populations in a serological study by Rieder *et al.*^[31]; strikingly lower cutoff points of gASCA, ACCA, ALCA, AMCA, anti-L, and anti-C were observed in children compared to adult patients with CD.

Pancreatic autoantibodies: Autoantibodies against exocrine pancreas (PAB) were described for the first time in 1984^[32], but the autoantigenic targets of PAB were identified only in 2009^[33,34]. The recognition of glycoprotein 2 (GP2) as a major target antigen of the droplet-like PAB (type I PAB) has been followed by the identification of CUB/zona pellucida-like domain-containing protein 1 (CUZD1) as another major antigenic target of PAB giving the reticulogranular, cytoplasmic pattern by indirect immunofluorescence (type II PAB). Both GP2 and CUZD1 are glycosylated membrane proteins residing in the acinar secretory storage granules of the pancreas. It was previously believed that GP2 is exclusively expressed by pancreatic acinar cells, but recent studies have shown that GP2 is also present as a specific membrane-anchored receptor on the microfold (M) intestinal cells of intestinal Peyer's patches, and is essential for host-microbial interaction and the initiation of bacteria-specific mucosal immune responses^[35,36]. Notably, GP2 overexpresses at the site of CD inflammation in contrast to UC^[33,37]. Respective data regarding CUZD1 expression in the intestine are sparse, with further research being needed to evaluate the relevance of these autoantibodies in CD. Combined determination of GP2 and CUZD1-specific autoantibodies by indirect immunofluorescence using recombinantly expressed human embryonic-kidney cell autoantigens represents a new method in the serological diagnosis of IBD. Discrimination between positive and negative reactions is considered to be easier in transfected cells than in primate tissues. The selective detection of anti-GP2 and CUZD1 autoantibodies by enzyme-linked immunosorbent assay (ELISA) has also been recently developed^[34].

PAB have been reported to be pathognomonic markers of CD. A prevalence of 27% to 39% of PAB was present in patients with CD, compared with only 0% to 5% in patients with UC^[38-40]. Increased prevalence of PAB has been found in unaffected first-degree relatives^[41]. Stöcker *et al.*^[38] reported that PAB could only be

determined in the serum of patients with CD. However, other studies found much higher (22%-24%) prevalence of PAB in UC^[42-44]. Although anti-GP2 only represents a small proportion of PAB seropositive cases, anti-GP2 autoantibodies are detected in about 30% of patients with CD and in 5%-12% of patients with UC^[45-47].

Ulcerative colitis

Autoantibodies against intestinal goblet cells: Serological markers have been far less extensively studied in UC than in CD. Autoantibodies against different colonic antigens have been found in patients with UC [*e.g.*, goblet cell autoantibodies (GAB)]. In previous studies, GAB has been detected in adult patients with UC, with a prevalence of 28% to 30%. In contrast, other studies suggested a much lower prevalence in both diseases^[42-44]. These conflicting results are likely due to methodological differences, such as enzyme-linked immunosorbent assay antigen substrates and the evaluation of fluorescence patterns. GAB produce mucin that has multiple functions: it serves as a lubricant, provides nonspecific protection against unwanted microbial agents, and hosts the normal bacterial flora. Through complicated and strictly regulated glycosylation, mucins act as a decoy in binding a range of different microbes and maintaining the normal intestinal flora. The significance of these antibodies, however, has not been established and thus remains unclear.

DIAGNOSTIC VALUE OF NEW SEROLOGIC MARKERS IN IBD

In diagnostic workup of IBD, a serologic test with high sensitivity and specificity is desired. The diagnostic value of the new serologic markers for IBD is limited due to their low sensitivity and presence in other conditions, such as celiac disease, autoimmune diseases, and liver cirrhosis^[48-50]. Sensitivity can be increased by the combination of different antibodies. A role for serological testing in screening for IBD was suggested by several studies, but the low sensitivity of these assays only provide a modest contribution to the identification of IBD^[8,24,51-53]. The diagnostic value of the new serologic markers in children with IBD is shown in Table 1. A retrospective study of 300 pediatric patients tested in the IBD7 panel (anti-OmpC, anti-CBir-1, ASCA, and ANCA, Serology 7, Prometheus, Sandiego, CA, United States) for the evaluation of pediatric IBD resulted in a 67% sensitivity and 76% specificity. Consequently, this panel has a limited clinical utility in screening for pediatric IBD^[53].

In pediatric CD, each anti-OmpC, anti-I2, or anti-CBir1 antibody was detected in 11%-55% of patients as a single marker. In a prospective pediatric study using combined analysis (anti-OmpC, anti-I2, anti-CBir1 or ASCA), 77% of patients with CD were positive for at least one microbial-driven antibody^[26]. Therefore this method provided modest support for the diagnosis of CD.

Single glycan markers have limited clinical value for the primary diagnostic workup for CD due to their low

Table 1 Diagnostic value of the new serological markers in children with inflammatory bowel disease

Marker	Sensitivity		Specificity	PPV	NPV	Ref.
	CD	UC		CD vs UC		
Anti-Omp	11%-34%	5%-25%	75%-95%	57.9%-69%	51.6%-53.3%	[8,24,25]
Anti-CBir	52%-56%	ND	ND	ND	ND	[10,13,26]
Anti-I2	44.4%-50%	41.7%-42%	58%-58.3%	51.6%-54.3%	51.1%-53.7%	[27,28]
gASCA	60.7%-62.7%	11.1%-14.6%	85.4%-88.9%	87.1%-92.5%	52.2%-55.9%	[30 ¹ ,31]
ACCA	8.7%-22%	3%-18.5%	81.5%-97%	72.2%-83.3%	32.4%-38.2%	[30 ¹ ,31]
ALCA	19.7%-30.5%	7.6%-14.8%	85.2%-92.4%	81.8%-81.6%	35.9%-40.1%	[30 ¹ ,31]
AMCA	12.2%-16.9%	7.6%-14.8%	85.2%-96.7%	71.4%-86.3%	31.9%-39.06%	[30 ¹ ,31]
Anti-L	18%-22%	3.3%-14.8%	85.2%-96.7%	76.5%-90.3%	33.3%-40.07%	[30 ¹ ,31]
Anti-C	10.2%-22%	2.3%-14.8%	85.2%-97.7%	76.5%-83.3%	33.3%-38.8%	[30 ¹ ,31]
PAB	34%-38.5%	20.4%-20.6%	79.4%-79.6%	62.5%-65.1%	54.7%-56.5%	[44,45] ¹
Anti-GP2	30.2%	8.8%	91.2%	77.4%	56.7%	[45] ¹
GAB	12.2%	1.9%	98.1% ²	86.5% ²	52.7% ²	[44]

¹Mixed pediatric and adult cohort; ²Ulcerative colitis (UC) vs Crohn's disease (CD). ND: No data available; Anti-OmpC: Antibodies against outer membrane porin C of *Escherichia coli*; Anti-CBir1: Antibodies against bacterial flagellin CBir1; Anti-I2: Antibodies against the *Pseudomonas*-associated sequence; ASCA: Antibodies against *Saccharomyces cerevisiae*; AMCA: Anti-mannobioside carbohydrate antibodies; ALCA: Anti-laminaribioside carbohydrate antibodies; ACCA: Anti-chitobioside carbohydrate antibodies; Anti-L: Anti-laminarin carbohydrate antibodies; Anti-C: Anti-chitin carbohydrate antibodies; PAB: Pancreatic antibodies; Anti-GP2: Antibodies against glycoprotein 2; GAB: Antibodies against intestinal goblet cells; IBD: Inflammatory bowel disease; PPV: Positive predictive value; NPV: Negative predictive value.

sensitivity. From the entire panel, gASCA came out as the most accurate for the diagnosis of pediatric CD (sensitivity: 62.7%, specificity: 95.6% CD vs controls, and 88.9% CD vs UC)^[31]. With respect to the latest two novel markers, the addition of Anti-L and Anti-C to gASCA and pANCA further improved discrimination between CD and UC ($P < 0.001$) in a large pediatric and adult cohort with IBD ($n = 818$, 517 CD, 301 UC)^[30]. More specifically, nearly three-quarters of the patients with CD showed seropositivity for at least one of the aforementioned seven anti-glycan antibodies^[30,31]. Anti-glycan antibodies may be particularly important in ASCA-negative patients with CD. Rieder *et al.*^[51] found that 40.9% of ASCA-negative pediatric patients with CD were positive for at least one other anti-glycan marker, suggesting that these novel antibodies may further improve serological diagnosis for CD. Similarly, other studies found that about half of ASCA negative adult patients were positive for ALCA, ACCA, or AMCA^[29,54]. In concordance with the results published by Rieder *et al.*^[55], Seow *et al.*^[30] demonstrated that all the anti-glycan antibodies were highly specific for IBD, particularly for CD (85.4%-97.7%), and were more prevalent in CD vs UC ($P < 0.0015$). In this large pediatric and adult cohort with IBD, anti-C showed the highest specificity of 97.7, followed by ACCA at 97%, then anti-L at 96.7%. Due to the combined use of these markers, the specificity for CD increases up to 100%^[29,55].

While the specificity of PAB for CD is high, its sensitivity is low. In our study the presence of PAB was significantly higher in CD (34%) and UC (20.4%) compared with the pediatric control cohort (0%, $P < 0.0001$). Specificity of PAB was 100%; however, sensitivity was low. The combination of PAB and antibodies against ASCA/pANCA improved the sensitivity of serological markers in CD (87.4%) and in UC (79.6%); specificity was 89.3% and 93.2%, respectively^[44]. Combinations of these antibodies, particularly with ASCA, have shown

increased sensitivity; therefore, it may be recommended in the diagnostic procedure of IBD^[42,44]. Diagnostic accuracy of the combined novel antibodies with conventional serological markers in children with IBD is shown in Table 2^[44].

In a recent study, Bogdanos *et al.*^[45] observed a significantly higher prevalence of PAB compared to anti-GP2 in UC (20.6% vs 8.8%, $P < 0.003$), whereas the difference between PAB and anti-GP2 did not reach a statistically significance level in CD (38.5% vs 30.2%, $P = 0.108$), respectively. Thus, anti-GP2 testing by ELISA assay seems to be more specific for CD than for PAB testing, so it may improve the differentiation between CD and UC.

In UC, the most frequently studied serological marker is pANCA. Besides pANCA, in our study the prevalence of GAB was significantly increased in patients with UC in comparison to CD and controls (UC, 12.2%; CD, 1.9%; controls, 1.9%; $P = 0.02$). Sensitivity can be significantly increased with combinations of different antibodies. For example, pANCA and/or GAB together had a sensitivity of approximately 80% for UC^[44].

ASSOCIATION WITH IBD PHENOTYPES AND PROGNOSIS

In patients with CD at diagnosis, most patients have inflammatory type disease^[56,57]. Nevertheless, during the disease course the development of complicated behavior in the pediatric population is a common feature^[58]. In the largest pediatric cohort with CD ($n = 989$), the cumulative incidence of stricturing or penetrating complications was found to be 13%, 27%, and 38%, 1, 5, and 10 years after the diagnosis of IBD, respectively^[58]. Furthermore, small bowel disease is more frequently correlated with the development of complicated disease behavior than in isolated colonic disease. Based on these observations,

Table 2 Diagnostic accuracy of the combined novel antibodies with conventional serological markers in children with inflammatory bowel disease^[44,45]

	Marker	Sensitivity	Specificity	PPV	NPV	Ref.
CD <i>vs</i> controls	ASCA	35.5%-72.8%	95.2%-96.5%	91%-93.8%	59.9%-77.8%	[44,45] ¹
	PAB	34.0%-43.8%	100%	100%	60.2%	[44,45] ¹
	Anti-GP2	30.2%	96%	88.3%	57.9%	[45]
	pANCA	33.0%	94.2%	85.1%	58.4%	[44]
	GAB	1.9%	98.1%	50.0%	50.0%	[44]
	PAB and/or ASCA	79.6%	95.2%	94.3%	82.3%	[44]
	Anti-GP2 and/or ASCA	50.9%	92.9%	87.8%	65.4%	[45]
	PAB and/or ASCA and/or pANCA	87.4%	89.3%	89.1%	87.6%	[44]
	PAB and /or ASCA/ pANCA-	53.4%	95.2%	91.8%	67.1%	[44]
	ASCA+/pANCA-	51.5%	95.2%	91.5%	66.2%	[44]
UC <i>vs</i> controls	pANCA	77.5%	94.2%	93.0%	80.9%	[44]
	GAB	12.2%	98.1%	86.5%	52.8%	[44]
	PAB	20.4%-23.5%	100%	100%	55.6%	[44,45] ¹
	Anti-GP2	8.8%	96%	68.8%	51.3%	[45] ¹
	ASCA	6.9%-26.5%	95.2%-96.5%	66.3%-84.7%	50.9%-56.4%	[44,45] ¹
	ASCA ²	16.3%	95.2%	77.3%	53.2%	[44]
	PAB and/or pANCA	79.6%	94.2%	93.2%	82.2%	[44]
	PAB and/or pANCA and/or GAB	79.6%	94.2%	93.2%	82.2%	[44]
	Anti GP2 and/or ASCA	14.7%	92.9%	67.4%	52.1%	[45]
	GAB+/pANCA+	12.2%	98.1%	86.5%	52.8%	[44]
	PAB+/pANCA+	18.4%	100%	100%	55.1%	[44]
	rPAB+/pANCA+	22.4%	100%	100%	56.3%	[44]
	GAB+/PAB+/pANCA+	4.1%	100%	100%	51.0%	[44]

¹Mixed pediatric and adult cohort; ²Diagnostic value of antibodies against *Saccharomyces cerevisiae* (ASCA) antibodies in ulcerative colitis (UC) patients without primary sclerosing cholangitis (PSC). PAB: Pancreatic antibodies; Anti-GP2: Antibodies against glycoprotein 2; pANCA: Perinuclear anti-neutrophil cytoplasmic antibodies; GAB: Antibodies against intestinal goblet cells; IBD: Inflammatory bowel disease; CD: Crohn's disease; PPV: Positive predictive value; NPV: Negative predictive value.

a more aggressive treatment should be considered in this large subgroup of pediatric patients with CD. Consequently, the evaluation of relevant phenotype-serotype correlations may provide important prognostic information. Association of the new serologic markers with phenotype in pediatric CD is summarized in Table 3.

Antibodies directed to bacterial antigens were reported as being qualitatively (presence) and quantitatively (titer) associated with aggressive disease behavior in both children and adults^[10,26,59,60]. The first prospective pediatric study conducted by Dubinsky and co-workers demonstrated that the degree of the immune response to ASCA, anti-I2, anti-OmpC, and anti-CBir1 correlated with internal penetrating, stricturing disease, and the need for surgery in a large cohort with CD ($n = 196$). The risk of developing penetrating and/or stricturing CD was increased 11-fold in those subjects with immune responses to all four antigens (anti-I2, anti-OmpC, anti-CBir1, and ASCA) compared to seronegative cases (OR = 11, 95%CI: 1.5-80.4, $P = 0.03$). Moreover, in this study, the highest antibody sum group and quartile sum score group showed the most rapid disease progression^[26]. These initial findings were confirmed in another larger study of 796 pediatric CD patients using ASCA, anti-OmpC, and anti-CBir1^[10].

Recent studies demonstrated that seropositivity for anti-glycan antibodies was associated with early disease onset, small bowel disease, complicated disease behavior, and CD-related surgery in both adult and pediatric

CD^[4,29,30,31,54,55,61,62]. This was also found in both qualitative (number of positive antibodies) and quantitative (antibody titers) immune response. In a cross-sectional pediatric study, ALCA and anti-L had the strongest association with complications^[31]. In this pediatric population, most of the anti-glycan markers, except for ACCA and anti-C, were associated with complicated disease behavior and ALCA with CD-related surgery. Only gASCA was associated with terminal ileal disease location. Surprisingly, gASCA was inversely correlated with early disease onset in this pediatric cohort^[31], but this link was found to be positive in adult CD^[4,55,63]. This difference may arise from the distinct nature of the intestinal immune system in children.

There are conflicting results related to the association between PAB and CD phenotype in adult cohorts. Increased prevalence of PAB was observed in patients with early onset of disease, and stricturing or penetrating phenotypes^[39,40,42,43,64]. Lakatos *et al*^[42] reported an association between PAB positivity, perianal disease, and EIMs. However, in our pediatric study, we found that the presence of PAB was not associated with disease phenotype in CD^[44]. It is difficult to compare the data of these studies, since age may affect localization and behavior as well.

In some studies, the relation between anti-GP2 and CD phenotype was also evaluated. In mixed pediatric and adult cohort with CD ($n = 169$), humoral autoreactivity to GP2 and ASCA applying ELISA has been reported to be associated with ileocolonic location, suggesting a

Table 3 Association of the new serologic markers with phenotype in pediatric Crohn's disease

Marker	CD phenotype	Ref.
Anti-OmpC	Complicated disease behavior	[10,26]
Anti-CBir1	CD-related surgery	
Anti-I2		
ASCA		
gASCA	Early disease onset	[30 ¹ ,31]
	Ileal disease location	
	Complicated disease behavior	
	Perianal disease	
	CD-related surgery	
ACCA	Complicated disease behavior CD-related surgery	[30] ¹
ALCA	Ileal disease location	[30 ¹ ,31]
	Complicated disease behavior CD-related surgery	
AMCA	Complicated disease behavior perianal disease	[30 ¹ ,31]
Anti-L	Ileal disease location	[30 ¹ ,31]
	Complicated disease behavior	
	Perianal disease	
	CD-related surgery	
Anti-C	Complicated disease behavior	[30] ¹
	Perianal disease	
	CD-related surgery	
Anti-GP2 with ASCA	Early disease onset	[45] ¹
	Ileal location	
	Complicated behavior	
	Perianal disease	

¹Mixed pediatric and adult cohort. Anti-OmpC: Antibodies against outer membrane porin C of *Escherichia coli*; Anti-CBir1: Antibodies against bacterial flagellin CBir1; Anti-I2: Antibodies against the *Pseudomonas*-associated sequence; ASCA: Antibodies against *Saccharomyces cerevisiae*; AMCA: Anti-mannobioside carbohydrate antibodies; ALCA: Anti-laminaribioside carbohydrate antibodies; ACCA: Anti-chitobioside carbohydrate antibodies; Anti-L: Anti-laminarin carbohydrate antibodies; Anti-C: Anti-chitin carbohydrate antibodies; Anti-GP2: Antibodies against glycoprotein 2.

role for GP2 as a receptor on M cells in intestinal Peyer's patches^[45]. Moreover, in this cohort, the presence of anti-GP-2 was associated with younger age at the onset of the disease (< 16 years), stricturing behavior, and perianal disease in CD^[45]. Similarly, Pavlidis *et al*^[46] demonstrated that patients with colonic CD do not show significant antibody reactivity against GP2 compared to those who had ileal localization; the site of GP2-rich M cells. However, a Belgian study by Op De Beéck *et al*^[65] did not find any association between anti-GP2 seropositivity and clinical phenotype in CD ($n = 164$) using the same ELISA.

In patients with UC, both anti-CBir1 and pANCA positivity correlated with the development of pouchitis after ileal pouch-anal anastomosis. In a study by Fleshner *et al*^[66], diverse patterns of reactivity to microbial antigens were manifested as different forms of pouchitis ($n = 238$, age range: 8-81 years). Anti-CBir1 positivity indicated acute pouchitis only in patients who have low-level pANCA expression, with increased incidence of chronic pouchitis only in patients who had high-level pANCA expression. In a meta-analysis by Singh *et al*^[67], the risk of chronic pouchitis after IPAA was higher in ANCA-

positive patients, but the risk of acute pouchitis was unaffected by ANCA status. These data had a significant influence on the patients' treatment in post-operative course. The studies could not demonstrate any association between the presence of GAB and clinical presentation, medical therapy, or need for surgery in patients with UC.

ASSOCIATION WITH THE RESPONSE TO THERAPY AND DISEASE ACTIVITY

Recent studies have highlighted the connection of serologic markers with biologic therapies. Previous studies demonstrated that ASCA signals do not predict response to anti-tumor necrosis factor (TNF)- α therapies in CD^[4,68]. Comparative findings were reported regarding the effect of biological agents in the behavior of anti-GP2 antibodies. Belgian investigators did not find a robust effect of infliximab and adalimumab in patients followed up for 6-44 mo^[65].

No association was detected between anti-glycan markers and the response to corticosteroids and disease activity in children with CD^[31]. Similarly, in our study, we could not find any association between serum antibodies of PAB, ASCA, and ANCA and response to therapy^[44].

Dubinsky *et al*^[69] reported that a combination of phenotype, serotype, and genotype is the best predictive model of non-response to anti-TNF α agents in pediatric patients. In this study, anti-OmpC, anti-CBir1, anti-I2, ASCA, and pANCA serum markers were analyzed. The most predictive model included the presence of three novel "pharmacogenetic" loci, the previously identified BRWD1, pANCA, and UC diagnosis ($P < 0.05$). The relative risk of non-response increased 15-fold when the number of risk factors increased from 0-2 to ≥ 3 ($P < 0.0001$)^[69].

Based on longitudinal analysis, the presence of antibodies in IBD is relatively constant during the disease course^[62,70]. However, the prevalence of ASCA, anti-OmpC, and anti-I2 has been found to be more frequent when the disease persists for a long time^[12,60]. Furthermore, disease activity, CRP levels, or response to corticosteroids does not appear to influence marker levels in longitudinal studies. Therefore, serial measurement of antibodies may not provide additional information for the evaluation of IBD^[31,70].

CONCLUSION

The correct diagnosis and classification of IBD as either CD or UC is essential for choosing the appropriate therapy. Combined application of the novel antibodies (PAB/GP2) with conventional serology markers (ASCA/pANCA) increased sensitivity. Therefore, the use of combinations may be advisable in the diagnostic work-up of selected cases. Moreover, childhood-onset CD often leads to complicated disease (stricturing or penetrating) with increasing prevalence in parallel to disease duration.

In CD, information gained from a serologic profile, both qualitatively and quantitatively, may help to determine the likelihood of a more severe phenotype. In addition, pediatric UC is associated with pancolitis and a higher risk of colectomy. In patients with UC, serologic markers are associated with the development of pouchitis after ileal pouch-anal anastomosis. With this knowledge, clinicians will be able to stratify patients regarding the risk of disease progression, create a personalized treatment strategy, and try to modify disease course, thus improving long-term prognosis. Further simultaneous prospective multicentric studies are needed to evaluate the exact prognostic role of serologic markers which may help in the individual therapeutic management of pediatric and adult IBD.

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Vitamin D improves inflammatory bowel disease outcomes: Basic science and clinical review

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Abstract

Vitamin D deficiency is commonly diagnosed among patients with inflammatory bowel disease (IBD). Patients with IBD are at risk of low bone density and increased fractures due to low vitamin D levels, long standing disease, and frequent steroid exposures; as a result, it is well established that vitamin D supplementation in this population is important. There is increasing support for the role of vitamin D in strengthening the innate immune system by acting as an immunomodulator and reducing inflammation in experimental and human IBD. The active form of vitamin D, 1,25(OH)₂D₃, acts on T cells to promote T helper (Th)₂/regulatory T responses over Th₁/Th₁₇ responses; suppresses dendritic cell inflammatory activity; induces antibacterial activity; and regulates cytokine production in favor of an anti-inflammatory response. Murine and human IBD studies support a therapeutic role of vitamin D in IBD. Risk factors for vitamin D deficiency in this population include de-

creased sunlight exposure, disease duration, smoking, and genetics. Vitamin D normalization is associated with reduced risk of relapse, reduced risk of IBD-related surgeries, and improvement in quality of life. Vitamin D is an inexpensive supplement which has been shown to improve IBD outcomes. However, further research is required to determine optimal serum vitamin D levels which will achieve beneficial immune effects, and stronger evidence is needed to support the role of vitamin D in inducing disease response and remission, as well as maintaining this improvement in patients' disease states.

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Key words: Vitamin D; Inflammatory bowel disease; Immune response; Inflammation; Cytokines; Supplementation

Core tip: There is support for the importance of maintaining normal vitamin D levels in inflammatory bowel disease (IBD) patients, demonstrated by its anti-inflammatory actions in the gut. A randomized controlled trial examined the impact of vitamin D supplementation on IBD outcomes and demonstrated a reduced risk of relapse in vitamin D-treated Crohn's disease patients. Furthermore, vitamin D₃ and active vitamin D have been shown to reduce clinical disease activity and improve quality of life in IBD patients. Normalization of vitamin D levels is also associated with a decreased risk for IBD-related surgery. This vitamin has therapeutic benefit in IBD patients.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the intestine that causes abdominal pain, diarrhea, and weight loss and includes two forms, Crohn's disease and ulcerative colitis^[1]. IBD reduces quality of life and may cause financial stress by increasing disability and decreasing the capacity for work^[1,2]. The disease is complex and etiology is not completely understood; however, IBD is associated with abnormal immune responses to the body's natural intestinal bacteria^[1,2], which activate the gastrointestinal immune system. It is expected that genetic and environmental interactions play a role in the susceptibility of IBD^[1,2]. As there is currently no cure for IBD, medical therapy remains the mainstay treatment for achieving and maintaining remission^[2].

It is well-established that vitamin D plays a critical role in improving bone health and is specifically important for patients who are at risk of low bone density. Low bone mineral density is more prevalent among patients with Crohn's disease and ulcerative colitis compared to healthy controls^[3]. Long standing disease, multiple steroid exposures, and low serum 25-hydroxycholecalciferol [25(OH)D3] levels are contributing factors to decreased bone mineral density and increased fractures in patients with IBD^[3-5]. Therefore, vitamin D supplementation is essential in this high-risk group. There is, however, growing support for non-traditional actions of vitamin D including anti-inflammatory, anti-proliferative, cell differentiation, and apoptotic effects^[6]. These effects have led to examination of vitamin D in the pathogenesis of autoimmune diseases such as IBD^[6]. This review will address the regulatory role of vitamin D on immune responses and describe its relevance in regards to inflammatory bowel disease.

VITAMIN D PHYSIOLOGY

Vitamin D metabolism

Vitamin D is present in two major forms. Vitamin D2 (ergocalciferol) is present in plants, yeast, and fungi^[6,7], while vitamin D3 (cholecalciferol) can be obtained from animal sources such as oily fish and egg yolk^[6-8]. Vitamin D3 is also synthesized endogenously in the skin upon ultraviolet light exposure. Sun light exposure to the skin results in a photochemical conversion of 7-dehydrocholesterol to pre-vitamin D, which then rapidly converts to cholecalciferol. This process is self-limiting to prevent toxicity^[6,8].

Vitamin D is fat soluble and absorbed in the small intestine along with dietary fat. After incorporation into chylomicrons, it is rapidly delivered into the venous circulation^[3,4]. It is then transported by vitamin D binding proteins (DBPs) to the liver where it is converted to 25(OH)D3 by hepatic 25-hydroxylase^[6,7,9]. 25(OH)D3 is inactive, however, it is the main circulating form of vitamin D and the best indicator of vitamin D status^[6,9]. Vitamin D is primarily stored in the liver as well as in adipose

tissue. Once saturation of these tissues occur, 25(OH)D3 is released to circulate in the blood^[4], where it is predominantly bound by DBPs and albumin, leaving little in the free form^[3,7]. DBPs transport 25(OH)D3 to the kidney, where it is converted to its active hormonal form, 1 α ,25-dihydroxycholecalciferol [1,25(OH)2D3], by the enzyme 25-hydroxyvitamin D3-1 α -hydroxylase^[6-9]. It can now act on its receptor, the vitamin D receptor, in many target tissues, including the intestine, kidney, and bone, thereby altering transcription of target genes^[10,11]. In the target tissue, 24-hydroxylase catabolizes 1,25(OH)2D3 and 25(OH)D3 into their inactive metabolites, which are then excreted as calcitroic acid in the urine^[3,8,10].

The rate limiting enzyme in the metabolism of vitamin D is 1 α -hydroxylase. This enzyme is tightly regulated by plasma parathyroid hormone (PTH) and 1,25(OH)2D3. Active vitamin D production in the kidneys is directed by PTH^[4,6,8,9], which upregulates transcription of CYP27B1, the gene encoding for 1 α -hydroxylase^[9]. This results in an increased production of 1,25(OH)2D3 in the kidney. In turn, 1,25(OH)2D3 takes part in a negative feedback loop to suppress the transcription of PTH and CYP27B1, thereby decreasing production of 1,25(OH)2D3^[9]. Simultaneously, 1,25(OH)2D3 induces 24-hydroxylase production^[8,9]. This is an autoregulatory mechanism to suppress the actions of 1,25(OH)2D3^[9]. An overview of vitamin D sources and metabolism is outlined in Figure 1.

Vitamin D receptor

The vitamin D receptor (VDR) plays an important role in how vitamin D exerts its biological effects. It belongs to a superfamily of nuclear hormone receptors and is specifically activated by 1,25(OH)2D3^[11,12]. In response to 1,25(OH)2D3 binding, VDRs regulate gene transcription, thereby producing specific proteins to carry out vitamin D3 biological activity. The DNA binding domain of the zinc finger recognizes vitamin D response elements (VDREs), which are specific DNA sequences on cell-targeted genes^[11]. 1,25(OH)2D3 binding results in the formation of the VDR and retinoid X receptor (RXR) heterodimer^[12]. This complex binds to VDREs and recruits large coregulatory complexes to these specific genes through the VDR transactivation domain^[11,12]. The activities of coregulatory complexes may include nucleosomal remodeling, selective chromatic histone modification, or RNA polymerase II recruitment and initiation. All of these activities work to enhance or suppress gene expression^[11].

Multiple tissues and immune cells express VDRs and the enzymes needed to produce local 1,25(OH)2D3^[6,13-15]. This enzyme activity is regulated in a different manner compared to the enzymatic renal production of 1,25(OH)2D3; it is no longer under an endocrine feedback mechanism, but is induced by other factors^[6]. These findings have led to the examination of multiple roles of vitamin D in the pathogenesis of autoimmune diseases such as IBD^[6].



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Overall, the main action of 1,25(OH)2D3 on T-cells is mediating T helper type 1 (Th1)/T helper type 2 (Th2) development and differentiation^[17,18]. Vitamin D3 affects

the Th1-Th2 balance in favour of Th2 cell development. The development into either Th1 or Th2 from CD4⁺ T cells is directed by cytokines^[18]. Cytokine IL-12 induces Th1 cell development whereas IL-4 induces Th2 cell development. The effects these cytokines have on the Th1-Th2 balance determines which cytokines will be produced and therefore determine the type of immune response. Th1 cells produce pro-inflammatory IFN- γ and lymphotoxin, and Th2 produces anti-inflammatory IL-4, IL-5, and IL-13^[17,18]. The increased development of Th2 is a result of direct action of vitamin D3 on CD4⁺ cells^[18], whereas the reduction in Th1 cell development is due to the effects of vitamin D3 on dendritic cells^[17,18]. Using mice Ag-specific T cells, Boonstra *et al*^[18] demonstrate that vitamin D3 increases the frequency of IL-4 producing cells *in vitro* from 8.0%-55.8%, thereby supporting a vitamin D induced Th2 profile. This study also shows that vitamin D3 increases IL-10 and IL-5 producing cells and decreases IFN- γ producing cells^[18]. The role of vitamin D in regulating cytokine production has been suggested to limit inflammatory tissue damage, but it also reduces bacterial killing. IFN- γ augments toll-like receptor (TLR) 2/1 induced expression of CYP27B1 and bacterial killing, and IL-4 depresses TLR 2/1 activation of bacterial killing^[13]. Therefore, a balanced response is important.

At the transcription level, c-maf and GATA-3 are the transcription factors relating to Th2 development^[18]. Vitamin D3 can directly target CD4⁺ cells to promote Th2 development at the level of transcription^[17,18]. *In vitro* studies show a correlation between increased expression of GATA-3 and c-maf and Th2 cytokine levels, IL-5, IL-10, and IL-4, after vitamin D3 treatment. In addition, there is a reduction in IFN- γ ^[18]. Boonstra *et al*^[18] conclude that vitamin D likely functions to diminish cell-mediated immune responses by skewing toward a Th2 phenotype, which fights extracellular infections.

Th17 cells are another vitamin D target because of their ability to produce IL-17, a pro-inflammatory cytokine. Vitamin D reduces inflammatory tissue damage by suppressing Th17 development and in doing so, reduces IL-17 production^[13]. Furthermore, 1,25(OH)2D3 increases the development of T regulatory cells by acting on naïve CD4⁺ cells. Regulatory T cells are a group of CD4⁺ T cells that have immunosuppressive properties by depressing the proliferation of other CD4⁺ T cells^[13]. Vitamin D in combination with another immunosuppressive drug, dexamethasone, has been shown to increase IL-10 producing regulatory T cells. This supports the idea that regulatory T cells located at sites of inflammation down-regulate the immune response^[19].

Research supports the immunosuppressive effect of vitamin D due to its involvement in T-cell differentiation. The evidence shows that vitamin D plays a role in maintaining a balance between the inflammatory response of Th1/Th17 cells and the immunosuppressive response

from Th2/Treg cells.

Vitamin D and dendritic cells

Dendritic cells (DCs) are antigen presenting cells (APCs) and are important in initiating CD4⁺ T cells responses^[20]. Vitamin D inhibits differentiation and maturation of human DCs *in vitro*^[20] by suppressing the IL-12 production from DCs and increasing IL-10 production^[17,20]. This is an important immunosuppressive activity as IL-12 is an important cytokine in inducing Th1 development^[17,18]. 1,25(OH)2D3 inhibits the differentiation, maturation, and immunostimulatory capacity of DCs^[21]. Canning *et al*^[21] confirm that 1,25(OH)2D3 suppresses monocyte differentiation into DCs, thereby generating immature DCs. This suppresses DC ability to stimulate T-cell proliferation.

Lipopolysaccharide (LPS) is a component of the Gram-negative bacterial wall, which induces monocytes/macrophages to produce cytokines. After LPS stimulation, DCs and monocyte-derived macrophages (MACs) have a high local 1- α -hydroxylase production of 1,25(OH)2D3, which positively modulates MAC differentiation and depresses actions of DCs and lymphocytes^[14]. This mechanism facilitates a normal immune response as some DCs still mature; vitamin D prevents over stress of this immune response that may lead to pathological effects^[13]. Vitamin D skews the development of the immune response towards a non-specific innate immune response and away from an antigen-specific immune response.

Antibacterial activity of vitamin D

As a fundamental part of the innate immune response, human monocytes have been shown to locally produce 1,25(OH)2D3, which triggers increased autophagy, an important mechanism for eliminating pathogens by antibacterial proteins^[15]. TLR expressed on monocytes recognize pathogens, and under TLR 2/1 stimulation, CYP27B1, the gene encoding for 1 α -hydroxylase, and VDR expression is upregulated^[13,15]. 1,25(OH)2D3 acts on monocytes to induce expression of the cathelicidin antimicrobial peptide (CAMP) gene (LL-37), producing a protein that enhances intracellular killing of bacteria^[13,15,22]. The CAMP gene is a direct target of the vitamin D receptor^[22]. Furthermore, nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor, has an important role in inducing antibacterial activity. NOD2 activation by muramyl dipeptide, a product of Gram-negative and Gram-positive bacteria, stimulates transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which induces gene expression for antimicrobial peptide defensin β 2 (DEFB2)^[13,23]. Interestingly, 1,25(OH)2D3 induces NOD2 expression in many cell types, thereby increasing cell sensitivity to these bacteria products; as a result 1,25(OH)2D3 enhances NOD2 mediated DEFB2 transcription^[13,23].

Anti-inflammatory role of vitamin D

As a part of the innate immune system, macrophages release proinflammatory cytokines and chemokines upon stimulation. This leads to an inflammatory response to protect the body from pathogenic microorganisms^[24]. TNF- α is a proinflammatory cytokine that is produced early in the course of inflammation by macrophages and lymphocytes. It is involved in autoimmune diseases, including IBD^[25]. Proinflammatory cytokines are positive for host defense, but overproduction leads to unresolved inflammation^[26]. Muller *et al*^[27] examine the effect of 1,25(OH)2D3 on LPS-stimulated human blood monocytes, and find it inhibits the release of IL-1 α , IL-6, and TNF- α . LPS binds to TLR4 on monocytes to mediate activation of mitogen-activated protein kinase (MAPK)^[26]. MAPKs are critical regulators of proinflammatory cytokine production, including IL-6 and TNF- α . 25(OH)D3 treatment has been shown to inhibit LPS-induced IL-6 and TNF- α production in peripheral blood mononuclear cells (PBMC) of healthy donors *via* upregulation of MKP-1 (MAPK phosphatase-1). MKP-1 switches off cytokine production in monocytes/macrophages after inflammatory stimuli. Interestingly, doses of 25(OH)D3 that relate to vitamin D sufficiency (> 30 ng/mL or 75 nmol/L) significantly inhibited mRNA production of these cytokines^[26].

Vitamin D analogues have also been proven to have immunomodulatory effects. Stio *et al*^[28] demonstrate the vitamin D analogue KH 1060 and a monoclonal anti-TNF- α antibody to work synergistically in significantly inhibiting PBMC proliferation and TNF- α production in healthy subjects. *In vitro* experiments using PBMCs from healthy volunteers were also able to demonstrate the effect of paricalcitol, a vitamin D analogue, in reducing both basal TNF- α and LPS-induced TNF- α ^[25].

The importance of VDRs in the inflammatory response has also been demonstrated. Chen *et al*^[24] report a more robust and prolonged production of pro-inflammatory cytokines TNF- α , IL-6, and IL-1 β in bone marrow derived macrophages (BMDMs) from VDR-/- mice compared to wild type (WT) mice after LPS exposure. This suggests a dysregulated and over sustained innate immune response in macrophages under attenuated VDR signalling. Interestingly, 1,25(OH)2D3 and its analogue, paricalcitol, have been shown to reduce LPS-induced TNF- α and IL-6 cytokines in WT BMDMs^[24].

Chen *et al*^[24] have also examined novel anti-inflammatory effects of vitamin D by investigating its microRNA-155-SOCS1 target. MicroRNAs (miRNAs) are small noncoding RNAs that control gene expression. Recently, miRNA-155 has been shown to regulate innate immune responses and TLR signaling. It targets the “suppressor of cytokine signalling” (SOCS) family of proteins, specifically SOCS1. miRNA profiling in mice cells was examined after the treatment with LPS, with or without 1,25(OH)2D3. As a result, miR-155 increased the most with LPS and was suppressed the most by vitamin D; miR-155 were elevated in VDR -/- bone marrow derived

macrophages (BMDMs) after exposure to LPS compared to the WT BMDMs; and 1,25(OH)2D3 suppressed the induction of miR-155 by LPS in these cells. Additionally, 1,25(OH)2D3 blocked TNF- α , IL-6, and miR-155 induction in human PBMCs. Overall, 1,25(OH)2D3 was found to upregulate SOCS1 through its suppression of miR-155. SOCS1 is important in the negative feedback regulation of LPS-induced inflammation and inhibits TNF- α , IL-6, and IFN- γ pathways. In the absence of VDRs, the negative feedback loop is dysregulated^[24].

Seasonal variations in serum vitamin D levels have an effect on the innate immune response. The increase in serum vitamin D levels during the summer months is associated with a significant drop in LPS-induced TNF- α (64%), IL-6 (33%), IL-1 β (59%), and IFN- γ (46%) from PBMCs in healthy individuals *in vivo* compared to LPS-induced levels during the winter months^[29]. Caution does need to be taken when considering the difference in physiological up-regulation of vitamin D levels by solar radiation and the doses of vitamin D employed *in vitro* and *in vivo*; however, this data does support how the innate immune response can be regulated by the physiological variation of serum vitamin D3 levels during the four seasons of the year^[29].

Role of vitamin D in gastrointestinal inflammation

There is support for the role of vitamin D in the immune system, particularly in reducing inflammatory responses. Recently, more studies have demonstrated the role for vitamin D specifically in gastrointestinal inflammation and suggest deficiency is associated with IBD.

The importance of vitamin D in reducing the pro-inflammatory profile of IBD patients is demonstrated by the impact of cytokine-induced apoptosis and cytokine disruption of epithelial barrier function. Epithelial apoptosis occurs as a normal physiological event in the gastrointestinal tract and the mucosal barrier function is still maintained; however, cytokine-induced apoptosis may disrupt the barrier, leading to abnormal mucosal permeability, a common occurrence among IBD patients^[30]. Bruewer *et al*^[30] address the mechanisms by which pro-inflammatory cytokines disrupt the barrier through non-apoptotic mechanisms. IFN- γ was demonstrated to reduce epithelial gate function, and the effects were increased when combined with TNF- α , resulting in intestinal epithelium paracellular permeability. The authors suggest this allows for the barrier function to quickly normalize when inflammatory cytokines are reduced^[30], stressing the importance of medical therapy in maintaining disease remission.

There is evidence to suggest that there are distinct cytokine profiles in Crohn's disease and ulcerative colitis. Papadakis *et al*^[31] report Crohn's disease to show a Th1 type of immune response with elevated IL-12, TNF- α , and IFN- γ cytokines, whereas ulcerative colitis presents with increased IL-5 secretion. Specific pro-inflammatory cytokines have been identified in the inflamed mucosa of Crohn's disease and ulcerative colitis patients such as

IL-1, IL-6, IL-8, and TNF- α ^[31]. Each of these cytokines upregulate the inflammatory cascade leading to more inflammation and tissue damage in the inflamed mucosa^[31]. Vitamin D has been shown to target these inflammatory pathways.

TNF- α is a central cytokine in the pathogenesis of inflammatory bowel disease^[31]. In the review by Papadakis *et al*^[31], they report three lines of evidence to support the importance of TNF- α in IBD. They explain that anti-TNF therapy has been very successful in treatment of IBD in humans and in animal models, and that a “Crohn’s like” phenotype is expressed in mice that over express TNF- α ^[31]. Interestingly, a vitamin D analogue has recently been shown to work synergistically with infliximab, an anti-TNF therapy used in the treatment of IBD, to reduce the cytokine TNF- α in human peripheral blood monocytes^[28]. Treatment with 1,25(OH)2D3 in the colonic tissue of IL-10 knock-out (KO) mice has also been shown to down-regulate TNF- α -associated genes in these mice^[32]. Furthermore, *in vitro* studies of CD4⁺ T-cells of healthy controls and patients with Crohn’s disease have shown that 1,25(OH)2D3 increases the production of anti-inflammatory cytokine IL-10 and decreases the production of pro-inflammatory IFN- γ , supporting a therapeutic role of vitamin D in IBD^[33].

Stio *et al*^[34] examine the effects of vitamin D on PBMCs from patients with active Crohn’s disease on infliximab, an anti-TNF therapy. After vitamin D treatment, PMBC proliferation decreased in both responders and non-responders, but to a greater extent in responders^[34]. Interestingly, vitamin D analogue treatment increased VDR expression in unresponsive patients and VDR levels did not change in responsive patients. The authors suggest infliximab induces VDR expression in the presence of vitamin D in unresponsive patients; as a result, there are differences in sensitivity to vitamin D between responders and non-responders. This suggests that VDR expression and PMBC proliferation may be useful indicators to predict response of Crohn’s disease patients to infliximab therapy^[34].

Studies have gone on to suggest that vitamin D deficiency and deficiency in its signaling pathways are contributing factors in the pathogenesis of IBD. Wu *et al*^[35] propose a mechanism by which vitamin D deficiency may cause IBD through the changes in vitamin D receptor signaling in autophagy homeostasis, including increases in TNF- α -induced autophagy. Another study by Wang *et al*^[23] examine the role of vitamin D in NOD2 expression, as NOD2 deficiency, due to mutations in its gene, has been linked to the pathogenesis of Crohn’s disease^[22]. These authors show that 1,25(OH)2D3 signaling induces NOD2 expression in human intestinal epithelial cells, thus supporting the idea that vitamin D deficiency plays a causative role in Crohn’s disease.

As discussed previously, local production of vitamin D is an important part of regulating microenvironment inflammatory profiles. DCs from healthy subjects convert 25(OH)D3 to the active form of vitamin D, 1,25(OH)2D3,

which could be important in local control of inflammation in Crohn’s disease patients^[36]. Bartels *et al*^[36] isolate peripheral monocytes from 20 Crohn’s disease patients who were vitamin D deficient, which were then matured into DCs *in vitro*. Addition of the active [1,25(OH)2D3] and inactive [25(OH)D3] vitamin D3 metabolites inhibited LPS-induced DC maturation, and supplementation changed the cytokine profile of LPS-matured DC cultures compared to those not supplemented. The production of TNF- α and IL-12 decreased, while unexpectedly, there was a trend towards reduced IL-10 and IL-6 significantly increased^[36]. DCs of Crohn’s disease patients could be exposed to LPS in the gut resulting in similar findings^[36]. Furthermore, vitamin D supplementation reduced proliferation of the entire lymphocyte population and the CD4⁺ lymphocytes; therefore, vitamin D could be protective against Crohn’s disease as inflammation in this disease can be characterized as uncontrolled lymphocyte development^[36].

While the addition of vitamin D *in vitro* changes cytokine profiles, there may be differences in the effects of vitamin D in *in vivo* studies. A study was conducted on Crohn’s disease patients who were treated with 1200 IU of vitamin D3 per day or placebo for 26 wk to assess differences in induced T-cell mediated immune function between the treatment and placebo groups during *in vivo*^[37]. PBMCs were cultured from patients of each group, and there was a significant increase in production of IL-6 in T cells from vitamin D treated patients. IL-6 significantly correlated with serum vitamin D [25(OH)D3] levels ($P = 0.02$). In the vitamin D treated patients, there was also a trend to increased IL-4 and no effect on TNF- α and IFN- γ production^[37]. There was no change in IL-10 production nor in the amount of T regulatory cells CD4⁺, CD25⁺, FoxP3⁺ T cells; however, the amount of proliferating CD4⁺ cells significantly increased. IL-6 can induce T cell IL-4 production, which may explain the increase in IL-4^[37].

There is strong evidence for a protective effect of vitamin D against IBD related inflammatory responses; however, there are mixed findings on the production of IL-6 with the treatment of vitamin D. IL-6 has been shown to increase in certain situations and decrease in others under vitamin D treatment. Interestingly, *in vitro* and *in vivo* studies examining vitamin D treatment of LPS-matured DCs and PBMCs from Crohn’s disease patients show increased IL-6 production, whereas these studies examining healthy volunteers demonstrate a decrease in IL-6 after vitamin D treatment. IL-6 has been reported to support Th17 development, which have important anti-microbial roles^[36]. Additionally, IL-6 may have anti-inflammatory roles by reducing DC maturation and increasing T cell IL-4 production^[36,37]. Therefore, vitamin D does have immunosuppressive properties; however, instead of isolating vitamin D to this effect, it may be better to suggest it is an immunomodulator that strengthens the innate immune response and depresses the adaptive immune reaction^[36], which may explain the

different actions in healthy volunteers and IBD patients who have intestinal mucosal injury.

EFFECT OF VITAMIN D IN ANIMAL MODELS OF IBD

VDR KO mice models

Vitamin D and the vitamin D receptor have been shown to have an important role in animal models of colitis. Vitamin D receptor KO mice treated with dextran sodium sulfate (DSS) to induce colitis demonstrate markedly elevated levels of a number of tissue pro-inflammatory cytokines including TNF- α , IL-12p70, and IFN- γ , and have significantly more intestinal injury, fewer intact crypts, more epithelium loss, and more inflammation. This indicates that the absence of VDRs increases the sensitivity of the intestinal mucosa to very low doses of DSS^[38], thereby increasing the susceptibility to chemical injury in the gut. This confirms a prominent role of VDR signalling in the regulation of gut inflammation^[38].

Oral intake of 1,25(OH)2D3 improves DSS induced colitis in WT mice, and there is an upregulation of anti-inflammatory cytokine IL-10 production, which may help to reduce other cytokine responses^[38]. Interestingly, rectal administration of 1,25(OH)2D3 in the WT mice is more effective than oral in regards to decreasing DSS colitis. This is justified by obtaining the same results but only injecting half the dose of vitamin D in the rectum (site of inflammation)^[38].

Vitamin D and its receptors play important roles in maintaining gut integrity and protecting the intestine from pathogenic enteric bacterial infection. Wu *et al*^[39] support the role of vitamin D in suppressing bacterial-induced NF- κ B activity in the intestine. NF- κ B is a regulator of inflammatory cytokines such as IL-6, and after bacterial invasion, VDRs inhibit its actions. Intestinal VDRs protect against bacterial infection, demonstrated by a 6-fold increase in IL-6 and more severe inflammation after a *Salmonella* in VDR-/- mice compared to VDR+/+ mice^[39]. Furthermore, after exposure to *Salmonella*, mice lacking VDRs had more *Salmonella* invasion of the intestine compared to WT^[39]. Interestingly, VDR expression increased in WT mice as a direct result of an enteric bacterial infection, indicating a role of VDR signalling pathway in responding to specific pathogenic bacteria. VDRs relocated from the surface of colonic epithelial cells to the surface of crypts as well as in the middle and bottom of the crypts after infection^[39]. Furthermore, IL-6 was undetectable in VDR+/+ mice and present in VDR-/- mice; as a result, this suggests that VDR-/- mice start with a pre-inflammatory state even before being infected^[39].

The intestinal epithelial layer contains a highly specialized immune system and physical barrier to protect against the invasion of pathogens. Intraepithelial lymphocytes (IELs) are found in the intestinal epithelial layer, and they are an important part of maintaining intestinal integrity^[40]. Specific IELs, CD8 $\alpha\alpha^+$ T cells, aid in the maintenance of gut health. VDR KO mice have fewer

CD8 $\alpha\alpha^+$ T cells in the gut, implying VDRs are important in regulating the growth and maintenance of CD8 $\alpha\alpha^+$ T cells^[40]. Moreover, VDR-/- mice have significantly reduced intestinal transepithelial resistance (TER) compared to VDR+/+ mice. TER is reduced when epithelial barrier function is compromised, making it a good indicator of this malfunction. Additionally, tight junctions and desmosomes are severely disrupted in VDR-/- colonic mucosa^[41]. The breakdown of the physical barrier that separates the host from its gastrointestinal microorganisms results in intestinal permeability and the development of IBD. These studies demonstrate prominent role of VDR signalling in maintaining the integrity of the epithelial barrier in the intestine and in its protection against IBD^[42].

Increased IL-17 production has also been reported in VDR null mice. Th17 cells are pro-inflammatory and have been associated with increased severity of IBD in animal models. VDR KO mice have significantly higher induced Th17 cell and IL-17 production compared to WT mice^[43]. Additionally, 1,25(OH)2D3-deficient T cells overproduce IL-17 compared with WT cells^[43]. Recombinase-activated gene 2 knockout mice lack mature T and B cells. When WT CD4⁺ T cells are transferred to VDR/Rag double KO mice, the colonic sections of these recipients show significantly more hyperplasia and inflammation compared to Rag KO recipients^[43]. There is an increased expression of IL-17 secreting cells in the gut and periphery of these double KO mice, suggesting VDRs on accessory cells direct Th17 development^[43].

Conversely, there are some limitations of vitamin D on host susceptibility to pathogens. While increased production of Th1/Th17 cytokines is related to the pathogenesis of Crohn's disease, Th1/Th17 responses are critical for the removal of many infectious bacteria^[44]. Examining the role of 1,25(OH)2D3 in *Citrobacter rodentium* (*C. rodentium*) infected mice, Ryz *et al*^[44] demonstrate that mice treated with 1,25(OH)2D3 have significantly increased scores for edema, goblet cell depletion, hyperplasia and infiltrating inflammatory cells compared to vehicle-treated mice^[44]. These mice also have a reduced number of Th17 T cells in their colons and decreased production of Th17 associated antimicrobial peptide TEG3 γ , which works as a host defense against infection with *C. rodentium*^[44]. This study demonstrates the potential negative consequences of vitamin D treatment. It can protect against Th17 cell driven damage, but it is important to note these responses are key in host defense against pathogens^[44].

IL-10 KO mice models

VDR/IL-10 double KO mice develop severe IBD involving all areas of the small intestine and colon and have the largest change in colon anatomy and inflammation compared to single VDR KO and IL-10 KO mice^[45]. WT mice do not express any cytokines and VDR/IL-10 double KO mice express two-three fold higher levels of IL-1 β , IL-2, IFN- γ , and TNF- α mRNA in their colons

compared to the single KO mice colons^[45]. IL-10 KO mice with normal VDR function experience a milder form of disease; thus, VDR deficiency seems to intensify IBD severity. Single VDR KO mice also express a milder form of disease; therefore, IL-10 and VDRs have a collective effect on disease severity^[45]. Furthermore, vitamin D deficiency in IL-10 KO mice enhances inflammation in the small intestine in comparison to vitamin D sufficient and vitamin D supplemented IL-10 KO mice^[46]. Evidently, vitamin D deficiency augments disease severity; however, dietary intake of vitamin D can be problematic, as few foods are high in vitamin D and weight loss is common in patients with IBD^[46]. Therefore, vitamin D supplementation to achieve and maintain sufficient levels is critical.

Trinitrobenzene sulfonic acid and DSS induced colitis mice models

Colitis can be induced in mice with treatment of trinitrobenzene sulfonic acid (TNBS). While treatment alone with calcitriol, a vitamin D analogue, significantly reduces colitis in acute TNBS colitis, treatment with both calcitriol and dexamethasone shows the best improvement in health^[47]. This is demonstrated by the study by Daniel *et al*^[47], which showed weight gain and improvement in macroscopic, microscopic, and immunological parameters of colitis after treatment of TNBS-induced colitis with the combined therapies. Calcitriol treatment reduced Th1 mediators and increased IL-4, thereby promoting the Th2 subset^[47]. Additionally, calcitriol upregulated IL-10 and enhanced regulator T cell function, specifically transforming growth factor beta, and FoxP3 levels. Calcitriol also decreased IL-12p70 and IL-23p19 expression, which are DC mediators. As a result, calcitriol downregulates the pro-inflammatory response of intestinal DCs and counteracts Th1 action^[47].

A vitamin D analogue has been shown to provide similar results. ZK156979 is a new low calcemic vitamin D analogue, and its use in treating TNBS-induced colitis in mice results in remarkable disease improvement. Daniel *et al*^[48] show a reduction in colitis-associated hypercalcemia and inflammation as a result of treatment of TNBS-induced colitis with this vitamin D analogue. Infiltration of inflammatory cells, neutrophils and lymphocytes, ulcerations, loss of goblet cells, and fibrosis in the colon were restored after vitamin D treatment^[48]. TNF- α and IFN- γ levels decreased, and the Th2 profile was induced with increased production of IL-4 and IL-10^[48]. This analogue effectively treats Th1 colitis.

DSS treated vitamin D deficient and sufficient mice both show increased expression of mRNAs for cytokines IL-1, IL-10, IL-17, and IFN- γ ^[49]. Both types of mice show severe ulceration, granulation, and inflammation; however, symptoms are exacerbated in vitamin D deficient mice^[49]. Vitamin D acts to protect DSS treated mice, as there is an increased expression of vitamin D activating enzyme CYP27B1 in these mice^[49] and CYP27B1 KO mice are more susceptible to colitis after DSS

treatment^[50]. Lagishetty *et al*^[49] examine the impact of vitamin D status on antibacterial activity in DSS-induced experimental colitis. The expression of an antimicrobial protein, angiogenin-4 (Ang4), decreased in vitamin D deficient mice^[49]. Ang4 has bactericidal activity in the intestine and a decreased expression resulted in a 50-fold increase of bacterial infiltration in vitamin D deficient mice^[49]. It is worthy to note dietary restrictions in these mice resulted in 25(OH)D3 concentrations less than 50 nmol/L, which is consistent with human parameters of deficiency. The authors note that the consequences of vitamin D deficiency resulted after treatment with DSS; therefore, vitamin D deficiency may be important after inflammation has occurred^[49]. On the other hand, the expression of Ang4 was associated with vitamin D status; therefore, the authors hypothesize impaired vitamin D status may be a predisposing factor for IBD due to the regulation of enteric bacteria^[49].

Although these animal results are limited in their application to humans as chemical induced colitis in mice may not fully be representative of human IBD forms, they are important in examining treatment and disease activity outcomes. Investigation into determining whether other mouse models better replicate human IBD will be important^[50]. The differences in results between models with impaired vitamin D status as opposed to deletion of VDRs or the *CYP27B1* gene expression are also important in determining IBD susceptibility^[50].

EFFECT OF VITAMIN D IN HUMAN IBD

Geographical distribution of vitamin D deficiency in IBD

Epidemiological evidence for a role of vitamin D in IBD is seen in the geographical distribution of disease, with higher incidences and prevalence in temperate climates and lower risks in persons living near the equator^[6,51,52]. Additional environmental, lifestyle, or genetic factors that have similar associations with geographical location may play a part in the association between sun exposure and IBD^[51]; however, this “north-south” gradient in the risk of Crohn’s disease and ulcerative colitis is likely explained by the variation in sun exposure, a major determinant of vitamin D levels^[51,52], thereby strengthening the role of vitamin D in IBD.

Vitamin D deficiency has been well described in IBD patients from all over the world with varying prevalence. In Ireland, 63% of Crohn’s disease patients had 25-OH vitamin D levels < 50 nmol/L; however, using the higher cut-off to define vitamin D deficiency (< 80 nmol/L), 90% of this Crohn’s disease cohort was vitamin D deficient^[53]. The frequency of vitamin D deficiency in Canada is approximately 8% (< 25 nmol/L) with an additional 22% having insufficiency (< 40 nmol/L)^[54]. A Japanese study found that 27.3% of Crohn’s patients were vitamin D deficient (< 10 ng/mL or < 25 nmol/L) compared to only 6.7% of controls^[55]. In an IBD cohort of children, adolescents, and young adults from Philadelphia and Pennsylvania, 16% of the Crohn’s disease patients enrolled were vitamin

D deficient (< 38 nmol/L)^[56]. In a cohort study of IBD patients in Wisconsin, 49.8% of patients had levels < 50 nmol/L with 10.9% having levels < 25 nmol/L^[57]. Lastly, in London, United Kingdom, 68% of the IBD cohort was vitamin D deficient (< 50 nmol/L)^[58].

Higher 25-OH vitamin D plasma levels, predicted on the basis of a validate regression model in the Nurses' Health Study, have been shown to be associated with a lower incidence of Crohn's disease and ulcerative colitis^[59]; therefore, obtaining and maintaining optimal vitamin D levels are important, and yet, cut-off values used to define vitamin D deficiency among studies are variable. It is, however, generally accepted that vitamin D deficiency is defined as a serum 25-OH vitamin D level < 75 nmol/L^[8,60].

Risk factors of vitamin D deficiency in IBD

Many risk factors for vitamin D deficiency in IBD have been reported. Seasonal variation is evident in most studies with lower levels seen in winter months^[53,54,56,58]. Lower vitamin D levels have also been associated with longer disease duration and smoking^[53,57]. Poor health outcomes such as the need for intestinal resection, a structuring disease phenotype, the need for oral corticosteroids within 3 mo of diagnosis, and a diagnosis of pancolitis in ulcerative colitis are more prevalent in severely vitamin D deficient patients (< 25 nmol/L) compared to those with normal levels (> 50 nmol/L)^[58]. Intestinal resection may be a risk factor as the prevalence of at least one intestinal resection is significantly higher in those with vitamin D deficiency than those with adequate levels ($P < 0.05$)^[58].

The role of ethnicity as a risk factor for vitamin D deficiency in IBD has also been examined. A one year prospective study was conducted to examine the association between vitamin D levels, ethnicity, and human IBD. A significantly higher percentage of South Asian IBD patients were vitamin D deficient (< 50 nmol/L) compared to Caucasians^[61]. For both Caucasians and South Asians with Crohn's disease, an inverse relationship was found between clinical disease severity and vitamin D levels. For all IBD patients, CRP levels were inversely related to vitamin D levels; however, none of these results reached a significant association^[61]. Chatu *et al*^[58] also examine ethnicity as a predictor of vitamin D deficiency in an IBD cohort. No differences were found in median vitamin D levels among Crohn's disease and ulcerative colitis patients; however, the median vitamin D level was significantly lower in non-Caucasians (Asian and Black) compared to Caucasians. The multivariate regression analysis showed a history of IBD related surgery and ethnicity to be independently associated with vitamin D deficiency in Crohn's disease and ethnicity alone to be independently associated with vitamin D deficiency in ulcerative colitis^[58]. In an IBD cohort of children, adolescents, and young adults from Philadelphia and Pennsylvania, deficiency was more prevalent among African American subjects, Crohn's disease patients with upper gastrointestinal tract involvement, and patients with a significantly greater

lifetime exposure to glucocorticoid therapy^[56]. Other risk factors may include decreased nutrition intake due to Crohn's associated anorexia^[56,62], fear of GI discomfort from dairy due to lactose intolerance, and active disease associated with decreased physical activity resulting in reduced sun exposure^[56].

Genetic variants in the VDR and DBP have been shown to be associated with increased risk of IBD^[63,64]. Analysis of the frequency of common genetic variants in the DBP have shown the DBP 420 variant Lys to be less common in IBD patients as compared to healthy controls ($P = 0.034$)^[63]. A meta-analysis found that VDR gene polymorphisms are associated with the susceptibility to IBD. The TT genotype of TaqI was associated with Crohn's disease in Europeans (OR = 1.23; 95%CI: 1.02-1.49)^[63]. This polymorphism is a base substitution resulting in two encodings of isoleucine instead of an amino acid change. It has been suggested to result in lower VDR mRNA levels and less vitamin D/VDR inhibition on immune activation^[64]. Additionally, this variant was significantly associated with IBD in males. Furthermore, an increased risk of ulcerative colitis in Asians was significantly associated with the ff genotype of FokI on the promoter region of the VDR (OR = 1.65; 95%CI: 1.11-2.45)^[64]. This was compared to the FF genotype in Asians. The fourth finding was associated with decreased Crohn's disease susceptibility if one was a carrier of the "a" allele (Aa + aa genotypes) of ApAI (OR = 0.81; 95%CI: 0.67-0.97)^[64]. These studies demonstrate a genetic role explaining the high prevalence of vitamin D deficiency among IBD patients.

Vitamin D and disease activity

There is strong evidence to support a high prevalence of significantly lower serum 25-OH vitamin D levels among the IBD population, which have been shown to correlate with increased disease activity. Vitamin D levels have been shown to correlate negatively with disease activity assessed by the Harvey Bradshaw score^[57,61,62,64] or Crohn's disease activity index (CDAI) score^[65]. Joseph *et al*^[66] show that Crohn's disease patients have significantly lower vitamin D levels than their age- and sex-matched controls who were patients diagnosed with irritable bowel syndrome^[66]. Predictors of vitamin D status in this study were disease activity and sun exposure. In regards to severity of disease, patients with mild disease had vitamin D levels similar to the controls, but vitamin D levels were significantly lower in patients with moderate-severe Crohn's disease^[66]. As expected, lower vitamin D levels were observed in patients who had jejunal involvement of their disease^[66]. This association has also been reported in patients with ulcerative colitis. Blanck *et al*^[67] conducted a cross-sectional study and reported a larger number of patients with clinically active disease, using the six-point partial Mayo index, in the vitamin D deficient group compared to the vitamin D sufficient group ($P = 0.04$). Patients were stratified based on their vitamin D levels as either vitamin D suf-

ficient, insufficient, and deficient, and there continued to be a trend towards more active disease as vitamin D levels decreased^[67]. Furthermore, in a retrospective review of South Asian patients with IBD, patients with vitamin D deficiency appeared to have a more aggressive disease course with 14% of deficient patients requiring surgical management^[68]; therefore, optimizing vitamin D status and assessing the relationship between vitamin D treatment and disease activity is important^[68].

Despite a high prevalence of vitamin D deficiency among IBD patients, serum vitamin D levels may not always be associated with disease activity. Hassan *et al*^[69] found no association between low vitamin D levels and increased disease activity in IBD patients. Vitamin D deficiency may also be explained by the increased risk of intestinal malabsorption among the IBD population, particularly if patients have undergone small bowel resections or use cholestyramine for postresectional diarrhea. Cholestyramine reduces bile acids, which are required for vitamin D absorption^[6]. It has been demonstrated that Crohn's disease patients with quiescent disease have on average a 30% decrease in their ability to absorb vitamin D in comparison to normal subjects after supplementation with 50000 IU of vitamin D₂^[70]. Furthermore, Suibhne *et al*^[53] report vitamin D deficiency to be common among Crohn's disease patients in clinical remission. Even in the summer, vitamin D deficiency among these patients continued to remain high (50%). About 40% of these patients were supplementing with vitamin D (200-400 IU), but it was not enough to maintain optimal vitamin D levels^[53]. The location of disease, disease activity, or prior resection may not be the only factors affecting vitamin D bioavailability^[70].

According to the previous studies, vitamin D status has been reported to be inversely correlated with disease activity^[57,61,62,64,67]. However, Abreu *et al*^[71] demonstrate a significant positive correlation between modified Harvey Bradshaw indices and 1,25(OH)₂D₃ levels in Crohn's disease patients taking corticosteroids. This correlation, however, did not exist in the patients who were not taking this drug^[71]. The increase in systemic 1,25(OH)₂D₃ may be a result of increased inflammation as a result of Crohn's disease^[71]. This can be explained by the expression of 1 α -hydroxylase in the intestine and increased expression of 1 α -hydroxylase in inflamed biopsies of Crohn's patients^[71]. The authors conclude that elevated 1,25(OH)₂D₃ is an additional risk factor for osteoporosis in this study population as they determine glucocorticoid use and high 1,25(OH)₂D₃ levels to be independent risk factors for low bone mineral density. They also suggest that 1,25(OH)₂D₃ may be a direct cause of bone loss or a surrogate marker for the type of intestinal inflammation leading to osteoporosis^[71]. The evidence from this review would support the latter. It should be noted that the previous studies measured 25(OH)D₃ to determine vitamin D status, not 1,25(OH)₂D₃ levels.

Vitamin D supplementation in IBD

Vitamin D supplementation has traditionally been recommended in patients with IBD for management of bone disease. There is now increasing evidence for the potential immunomodulatory effects of supplementation. To date, the optimal level of 25-OH vitamin D for immunomodulatory effects is not known^[6].

Ananthakrishnan *et al*^[72] demonstrate an increased risk for IBD-related surgery in patients who have low plasma 25(OH)D levels. This association was found to be similar in both patients with Crohn's disease and ulcerative colitis. Furthermore, Crohn's disease patients who initially had a low level, which then was normalized, were significantly less likely to undergo surgery in comparison to patients who continued to maintain a low vitamin D level^[72]. A significantly lower C-reactive protein was also seen in these "normalized" patients. There was no association seen in ulcerative colitis patients, which the authors suggest may be due to a higher plasma 25(OH)D threshold in these patients to obtain any immune effects. Another explanation may be that vitamin D has a stronger interaction in Crohn's disease *vs* ulcerative colitis^[72]. Furthermore, Zator *et al*^[73] report a significant association between earlier cessation of anti-TNF therapy in IBD patients who had insufficient vitamin D levels prior to initiation of anti-TNF therapy, suggesting vitamin D may be an important adjuvant treatment aiding in the maintenance of response to this therapy. These studies denote the importance of repleting and maintaining sufficient vitamin D levels in patients who have IBD, specifically above 30 ng/mL (75 nmol/L), to reduce the risk of flares and to maintain response to IBD-therapies^[72,73].

One randomized placebo-controlled study has assessed the effectiveness of vitamin D supplementation in improving Crohn's disease activity. In comparison to the placebo, oral vitamin D supplementation of 1200 IU in adult patients with Crohn's disease in remission was shown to increase the 25-OH vitamin D levels and reduce the risk of relapse from 29% to 13% at 1 year ($P = 0.06$)^[74]. Although this difference in relapse was not statistically significant, the difference is clinically meaningful and does warrant further study. The authors did discuss the risk of type II error as an explanation for not reaching statistical significance^[74,75].

A prospective study completed by Miheller *et al*^[76] compares supplementation with active vitamin D (alfacalcidol) to non-active vitamin D (cholecalciferol) in Crohn's patients. Looking at the clinical course of Crohn's disease at 6 wk, disease activity significantly decreased in the active vitamin D group; however, there was no difference by the end of the trial at 12 mo^[76]. Active vitamin D treatment resulted in a significant decrease in CDAI scores and CRP levels, as well as improvement in quality of life scores. It has prominent short-term effects and may be due to improved immune responses^[76].

A systematic review^[75] was completed to examine the efficacy of vitamin D supplementation for treating colitis

Table 1 Vitamin D supplementation improves disease activity and outcomes in human inflammatory bowel disease

Ref.	n	Methodology	Aims	Intervention	Definition of improvement	Conclusions
Miheller <i>et al</i> ^[76]	37	Prospective single cohort study	Compare the effects of active vitamin D and plain vitamin D on bone health and disease status in Crohn's disease patients	Group A: 2 µg × 0.25 µg alfacalcidol (active vitamin D) daily Group B: 1000 IU cholecalciferol daily (plain vitamin D)	Assessment: Osteocalcin (OC) and beta-CrossLaps (βCL) concentrations CDAI, CRP, IBD-questionnaire (IBD-Q) Improvement: Significant decrease in OC and βCL concentrations Significant decrease in CDAI and IBDQ scores and CRP concentrations	Significant reduction in βCL and OC concentrations showed decelerated bone resorption and bone turnover in the active vitamin D group compared to the plain vitamin D group at 6 wk and 3 mo (<i>P</i> < 0.05) Significant reduction in disease activity and improved quality of life in the active vitamin D group at 6 wk (<i>P</i> < 0.05) No difference at 12 mo
Jørgensen <i>et al</i> ^[71]	94	A multi-centre randomized double blinded placebo controlled trial	Assess the efficacy of vitamin D supplementation in reducing the risk of relapse in Crohn's disease patients compared to placebo	Treatment group: 1200 IU vitamin D3 + 1200 mg calcium/d Placebo group: placebo + 1200 mg calcium/d	Assessment: CDAI Improvement: Decreased proportion of patients who achieve a CDAI score of 150+ and a 70 point increase in CDAI compared to baseline	Decreased risk of relapse (29% to 13%) at 1 yr with vitamin D treatment, but did not reach significance (<i>P</i> = 0.06)
Ananthakrishnan <i>et al</i> ^[72]	3217	Retrospective study	Assess the association between plasma 25(OH)D3 levels and IBD related surgeries and hospitalizations Assess changes in these outcomes after normalization of IBD patients' vitamin D levels	None	Risk of IBD-related surgery or hospitalization (OR); Improvement: Reduction in risk (OR < 1) for surgery or hospitalization	Low levels of vitamin D significantly increased risk for IBD-related surgery and hospital admissions (OR = 2.05; 95%CI: 1.53-2.75 for Crohn's disease and OR = 1.75; 95%CI: 1.21-2.52 for ulcerative colitis) Achieving normal vitamin D levels decreased risk of Crohn's disease-related surgery (OR = 0.56; 95%CI: 0.32-0.98)
Yang <i>et al</i> ^[77]	18	Prospective clinical pilot study	Establish the oral dose of vitamin D3 required to achieve serum 25(OH)D3 concentrations above 40 ng/mL (100 nmol/L) in mild-moderate Crohn's disease patients Assess improvement in disease activity and quality of life after vitamin D supplementation in Crohn's disease patients	Initiated on 1000 IU per day of vitamin D3 for 2 wk. Increased dose every two wk by 1000 IU/d until achievement of serum 25(OH)D level of 40 ng/mL occurred or patients were taking a total of 5000 IU/d	Assessment: Dose of vitamin D3 and serum 25(OH)D3 levels CDAI and IBDQ Improvement: Increase in serum 25(OH)D3 levels Reduction in CDAI scores of > 70 points or achievement of CDAI score < 150, and increase in IBDQ scores	Vitamin D supplementation improved vitamin D status at 24 wk (<i>P</i> < 0.001). 78% of patients required 5000 IU/d of vitamin D3, suggesting this is an effective dose in raising serum 25(OH)D3 levels in mild-moderate Crohn's patients Vitamin D treatment significantly improved disease activity and quality of life at 24 wk (<i>P</i> < 0.001)

CDAI: Crohn's disease activity index; CRP: C-reactive protein; IBD: Inflammatory bowel disease.

in humans and animals. With a primary outcome of inducing or maintaining remission of the disease, the above study by Miheller *et al*^[76] was included. The authors conclude that the inability to sustain differences in clinical outcomes with active vitamin D does not undermine the efficacy of vitamin D^[75]. It shows the additional improvement of the active form to the plain form. In comparison, Yang *et al*^[77] assessed the improvement in vitamin D status, clinical disease activity, and quality of life scores in 18 mild-moderate Crohn's disease patients who underwent vitamin D3 supplementation for 24 wk. Patients were started with 1000 IU/d of vitamin D3, and the dose was increased every two wk by 1000 IU until serum 25(OH)D3 levels were above 40 ng/mL (100 nmol/L) or the patients were taking 5000 IU/d. After 24 wk, the maximum dose of 5000 IU/d was required by 78% of patients and effectively raised serum 25(OH)D levels. The assessment of CDAI scores at 24 wk showed that 78% of patients achieved clinical response defined by a decreased CDAI score of 70 points or more. Additionally, 67% of patients were in remission and disease-specific quality of life significantly improved^[77]. Table 1 outlines the four studies examining vitamin D supplementation on IBD outcomes.

In a randomized clinical trial of children and adolescents with IBD, weekly high dose vitamin D2 (50000 IU) or daily vitamin D3 (2000 IU) were superior to daily vitamin D2 (2000 IU) in raising the serum 25-OH vitamin D level at 6 wk (25.4 ± 2.5 , 16.4 ± 2.0 , 9.3 ± 1.8 ng/mL, respectively)^[78]. Adherence may be improved with large doses, and dosing according to individual levels may achieve target levels most effectively^[3]. Recent studies have suggested that optimal targets for serum 25-OH vitamin D levels are greater than 30 ng/mL (75-80 nmol/L), with levels between 21-29 ng/mL (51-74 nmol/L) being defined as insufficient and levels < 20 ng/mL (< 50 nmol/L) being defined as deficient^[60].

CONCLUSION

Recent advances in the understanding of the effects and mechanism of action of vitamin D on the mucosal and systemic immune system and subsequently on intestinal inflammation suggests it has a role to play in the therapeutic management of IBD. Furthermore, both epidemiologic and emerging retrospective and prospective clinical evidence supports a significant beneficial role of vitamin D supplementation in patients with IBD. While the precise level of 25(OH)D3 that needs to be achieved for these therapeutic effects is unknown, it has been established that levels of 75 nmol/L or higher are generally adequate^[3,8,26,60,72]. Given the safety profile and low cost of vitamin D, its addition to the therapeutic armamentarium as a supplement to induction and maintenance therapy should be strongly considered.

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***Helicobacter pylori* infection and inflammatory bowel disease: Is there a link?**

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Abstract

Helicobacter pylori (*H. pylori*) infection is one of the most widely spread infectious diseases in humans. It can cause chronic gastritis, peptic ulcer disease and gastric malignancies and has been associated with extra-gastric disorders. *H. pylori* elicit a chronic systemic inflammatory response which, under certain conditions, may trigger autoimmune reactions and may be implicated in the pathogenesis of autoimmune diseases. Although the pathogenesis of inflammatory bowel disease (IBD) is unknown, it is thought to result from complex interactions between environmental factors and microbiota in the gut of individuals who are genetically susceptible. Several bacterial and viral agents have been implicated in the aetiology of IBD. In theory, *H. pylori* infection could be involved in the pathogenesis of IBD by inducing alterations in gastric and/or intestinal permeability or by causing immunological derangements resulting in absorption of antigenic material and autoimmunity via various immunological pathways. Similar mechanisms may also be responsible for the co-existence of IBD with other autoimmune diseases and/or extra-intestinal manifestations. However, the epidemiological data fail to support this association. In

fact, various studies indicate that the prevalence of *H. pylori* infection is low in patients with IBD, suggesting a protective role for this infection in the development of IBD. In this report, we aim to shed light on proposed mechanisms and confounding factors underlying the potential link between *H. pylori* infection and IBD.

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Key words: *Helicobacter pylori*; Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Colorectal cancer

Core tip: By gathering a large volume of published data, this review attempts to shed light on the mechanisms and confounding factors underlying the potential link between *Helicobacter pylori* (*H. pylori*) infection and Inflammatory Bowel Disease (IBD). However, whether the link between *H. pylori* and IBD is coincidental, epiphenomenal or mechanistic remains to be elucidated as there are contradictory data regarding both the causative and the protective role of *H. pylori* infection against IBD. This review provides a tool for researchers in this field to use as they perform further research to find the missing links.

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INTRODUCTION

Inflammatory bowel diseases (IBDs), which includes Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing-remitting diseases that constitute a

growing worldwide health burden^[1-3]. Over time, these diseases may lead to intestinal damage, complications, surgical interventions, gut failure and/or disability^[4-7]. IBD is thought to result from complex and unidentified interactions between environmental factors (such as infections, medicines, tobacco, food particles) and genetic factors of the host, resulting in abnormal and/or inappropriate immunological reactions to elements of the intestinal flora. For example, Gradel *et al.*^[8] demonstrated that infection with either *Campylobacter* or *Salmonella* species predisposed individuals to subsequent development of IBD.

Helicobacter species easily colonize the gastrointestinal surface due to microaerophilic metabolism, spiral shape, and peculiar motility^[9]. Based on their location within the gastrointestinal system, they are divided into gastric *Helicobacters*, such as the *Helicobacter pylori* (*H. pylori*), and enterohepatic *Helicobacters* (EHH), which predominantly colonize the intestine and the hepato-biliary system and have been linked to chronic liver and intestinal diseases^[9]. *H. pylori* usually resides in the surface epithelium of the stomach, but *H. pylori* DNA has also been identified in both the colon^[10] and stool of infected patients^[11-13].

H. pylori is a gram-negative, spiral-shaped pathogenic bacterium that causes chronic gastritis. Peptic ulcer disease and/or gastric malignancies may develop in a small number of individuals infected with the bacterium^[9,14]. The inflammatory response of the gastric mucosa to *H. pylori* most likely reflects the combined effects of a cellular immune response that is driven by an on-going stimulation of the host's immune system by the bacterium. This results in high production of interleukin (IL)-12, leading to a T helper type 1 (Th1)-polarized response and elevated levels of Th1 cytokines^[15-18]. Products of the local immune reactions may travel to extra-gastric sites, thus linking *H. pylori* infection to the pathophysiology of a variety of extra-gastric diseases, including autoimmune disorders^[19-21]. Interestingly, however, *H. pylori* has been proposed to play a protective role against the development of certain autoimmune disorders^[21] such as asthma^[22] and type 1 diabetes mellitus^[23]. The mechanisms underlying this protective role of *H. pylori* infection is thought to be differential expression of an acute and/or chronic local mucosal inflammatory response, which may elicit a systemic release of cytokines^[24], which in turn may down-regulate systemic immune responses and suppress autoimmunity.

In IBD, dysregulation of the immune response of the host to commensal bacteria has been proposed as an important underlying pathogenetic mechanism. Increased attachment of gut bacteria to the intestinal epithelium has been documented in IBD. A Th1 immune reaction and secretion of pro-inflammatory cytokines is implicated in the pathogenesis of IBD, especially CD^[5]. Up-regulation of cell signalling molecules, such as the macrophage inflammatory protein 3a (MIP-3a), has also been documented in IBD^[25]. Some IBD patients suffer from concomitant autoimmune diseases and autoimmune-type extra-intestinal manifestations. The similarities between

the immunobiology of IBD and that of *H. pylori* infection provides background for the hypothesis that *H. pylori* infection may be implicated in the pathogenesis of IBD.

Nevertheless, there is epidemiological evidence that contradicts the association between *H. pylori* and IBD. *H. pylori* infection is an infection that occurs in underprivileged societies and its prevalence declines when environmental hygienic conditions improve. In contrast the prevalence of IBD increases in societies adopting a Western life style^[26]. Thus, it appears that there is an inverse relation between the prevalence of IBD and *H. pylori* infection. IBD is highly prevalent in the United States^[27], an area with low rates of *H. pylori* infection whereas, a steady rise in the incidence of IBD has been observed in *H. pylori* endemic regions following widespread use of therapeutic regimens to treat *H. pylori*^[28]. Although environmental changes may be the confounding factor underlying this inverse relationship, many (but not all) epidemiological studies have shown a low incidence of *H. pylori* infection in patients with IBD^[29-60]. This has led to the hypothesis that *H. pylori* infection may exert a protective role against IBD. However one can argue that it is the medication used to treat IBD that eradicates *H. pylori* and /or the IBD associated mucosal alterations that may prevent colonization of the stomach by *H. pylori*. The latter may be true, especially in IBD patients with focally enhanced gastritis (FEG) who have a particularly low incidence of *H. pylori* infection, even if they live in *H. pylori* endemic areas^[53,54,61-64].

Possible mechanisms of the potential protective role of *H. pylori* infection against the development of IBD may be alteration of the host immunologic response away from the pro-inflammatory Th1/Th17 response towards an increased T-regulatory cell immune response^[65,66]. Moreover, *H. pylori* may induce the production of antibacterial peptides that counteract potentially harmful bacteria implicated in the pathogenesis of IBD^[67] or compete with bacteria for the same ecologic niche in the upper gastrointestinal tract^[68].

CAUSAL ASSOCIATION OF *HELICOBACTER* SPECIES AND *H. PYLORI* WITH IBD

In animal models, EHH such as *Helicobacter hepaticus* (*H. hepaticus*) and *Helicobacter bilis* (*H. bilis*) have been shown to induce a persistent inflammation in the colon and cecum in immuno-deficient rodents^[69,70]. *Helicobacter hepaticus* triggers colitis in a specific pathogen-free IL-10-deficient mice through an IL-12 and interferon-gamma (IFN- γ) dependent mechanism^[69]. *Helicobacter muridarum* increases disease activity and inflammation in an acute colitis model^[71] and provokes a CD-like inflammation in severe combined immunodeficiency mice upon receipt of T cells^[72]. Accumulating evidence from gene knockout rodents also indicate that the presence of EHH worsens the severity or hastens the development of colitis^[73,74].

However, observations from human studies are con-

flicting. Several EHH species have been identified in the large intestine of patients with enteritis and / or proctitis^[75]. *Helicobacter macacae* has been linked with chronic idiopathic colitis in young rhesus monkeys^[76]. Similarly, Laharie *et al.*^[77] found that *Helicobacter pullorum* (*H. pullorum*) or *Helicobacter canadensis* infection was significantly associated with CD in adults. *Helicobacter* species were found either in faecal specimens^[78] or in colonic biopsy samples^[79] of children with CD, and the prevalence of the *Helicobacteraceae* was significantly higher in children with CD (32/77, 41.5%) compared to controls (23/102, 22.5%)^[80]. A German group found *Helicobacter fennelliae* and *H. pullorum* in colonic samples of 12% of CD patients^[81]. *Helicobacter* genus PCR positivity was also significantly higher in UC than in controls (32/77 *vs* 11/59, *P* = 0.004)^[82]. *H. pylori* was isolated and detected by PCR in the intestinal mucosa of patients with UC-like CD and UC^[53,54,83]. Moreover, in another study *H. pylori* was found in faecal specimens in the majority of children with CD^[78]. In contrast, *Helicobacter* species were not detected in colonic biopsies of IBD patients in various studies^[84-88]. Additionally, no significant difference was observed in the rate of detection of *Helicobacter* species in intestinal biopsy specimens from 160 Chinese IBD patients (10%) and 80 controls (6.3%)^[57]. Furthermore, in an earlier study assessing gastrointestinal mucosal lesions in children with IBD, infection with *H. pylori* was found in only 2 of 41 children with CD (4.8%) and in 5 of 47 with UC (10.6%)^[89].

H. PYLORI AND THE NATURAL HISTORY OF IBD

It is conceivable that *H. pylori* infection may influence the clinical course of CD by triggering both specific and nonspecific immune responses in the human intestine. Phenotype modification of CD was identified in a study in which seropositive non-smoking CD patients had significantly fewer relapses and a lower risk of bowel resection compared to seronegative non-smoking patients^[90]. Moreover, serum anti-*H. pylori* IgG levels were significantly lower in subgroups of patients with fibro-stenotic and fistulising CD^[54].

There are several hypotheses regarding how *H. pylori* may influence the host immune response and thus alter the clinical course of CD. *H. pylori* infection may exert a direct damaging effect via urease and cytotoxins on the ileal or colonic mucosa^[91]. Moreover, *H. pylori* may induce an autoimmune-like reaction in the stomach with the production of anti-Lewis X and/or Y antibodies that have systemic auto reactive properties, thereby influencing the course of the disease^[92]. Another mechanism could be the induction of platelet activation and aggregation as shown in murine gastric venules which can cause the formation of microthrombi in gastric and intestinal epithelium and lead to infarction and development of ulcers^[93]. Another possibility is that *H. pylori* influences the host immune response via activation of the mucosa-associated lymphoid tissue (MALT), which may lead to a more generalized

immune response to *H. pylori* infection in IBD, contributing to the initiation or perpetuation of inflammation. In fact, Duchmann *et al.*^[94] showed that bacteria-specific T cell clones are increased in inflamed intestinal mucosa of patients with IBD.

It appears that in *H. pylori* infected patients, CD is more often confined to the terminal ileum, a location that is frequently affected by complications, yet may be associated with a lower clinical disease activity^[95]. *H. pylori* infection usually occurs early in life, before the onset of CD, so it is possible that this early infection may influence disease location in these patients^[96]. As a result, *H. pylori* infection may not influence the course of the disease primarily, but may influence the location of the disease and thus secondarily alters its course.

PROTECTIVE ROLE OF H. PYLORI AGAINST IBD

Many studies have reported that the prevalence of *H. pylori* infection is lower in patients with IBD compared to controls, demonstrating an inverse relationship between IBD and *H. pylori* infection that suggests a protective role of *H. pylori* infection against the development of IBD (Table 1)^[32-34,38,39,41-46,48,49,52,55,57-59]. However this has not been confirmed by other studies (Table 1)^[30,35-37,53,56,60]. Väre *et al.*^[41] found that seropositive CD patients presented at a significantly later age (40 years) compared to seronegative patients (30 years, *P* < 0.001), suggesting that the higher age of disease onset in seropositive IBD patients is the result of a protective modifier effect that *H. pylori* infection exerts on the development of IBD^[41], although this has not been confirmed by other studies^[34,42]. Furthermore, a meta-analysis of 23 studies suggested a protective role of *H. pylori* infection in CD pathogenesis, but the heterogeneity among enrolled studies and the possibility of publication bias limited the reliability of these results^[97]. The published literature on the prevalence of *H. pylori* infection in UC and CD is diverse. Various studies have found a lower prevalence of this infection in CD compared to UC^[29,31,34,41-43], whereas others have found exactly the opposite^[34,35,55]; still others have reported no difference in the occurrence of *H. pylori* between the two diseases^[30,32,33,36,37,39,48,56,57].

Moreover, the increased occurrence of *H. pylori*-negative FEG among IBD patients also confirmed the inverse association between the prevalence of *H. pylori* infection and IBD (Table 2). For example, *H. pylori*-negative chronic active gastritis was found in only 2% of patients without IBD compared to 20% of patients with IBD (CD 26%, UC 13%)^[98]. Furthermore, permanent colonization of the stomach by *H. pylori* is unusual in children with IBD^[40].

Heterogeneity among studies regarding the method of IBD and *H. pylori* diagnosis differences in study population, ethnicity and age across studies, and the possibility of publication bias may limit the certainty of the above findings. As environmental hygiene and intestinal

Table 1 Prevalence of *Helicobacter pylori* infection in patients with inflammatory bowel disease in different populations

CD	UC	C	Control group	Method	Positive (%)	Country	Ref.
42	51	40	Patients with irritable bowel syndrome	UBT, <i>H. pylori</i> IgG (+)	IBD: 17.2, C: 25 CD: 11.9, UC: 21.6	United Kingdom	[29]
110	213	337	Non-IBD patients with elective surgery ¹	<i>H. pylori</i> IgG (+)	IBD: 34.2, C: 36.2 CD: 33.3, UC: 34.7	United Kingdom	[30]
139	137	139	patients with functional GI disorders ¹	<i>H. pylori</i> IgG (+)	IBD: 9.4, C: 16 CD: 5, UC: 14	United Kingdom	[31] ²
47	63	100	Blood donors ¹	<i>H. pylori</i> IgG (+), UBT, histology	IBD: 21.8, C: 52 CD: 14.9, UC: 27,	United Kingdom	[32] ²
67	41	43	Non-IBD patients	Biopsies	IBD: 28.7, C: 39.5 CD: 28.4, UC: 29.3,	Italy	[33] ²
123	93	216	Blood donors ¹	<i>H. pylori</i> IgG (+), histology	IBD: 48.1, C: 58.8 CD: 40.7, UC: 55.9	Italy	[34] ²
32	40	72	Healthy subjects ¹	UBT	IBD: 47.2, C: 61.1 CD: 53.1, UC: 42.5	Italy	[35]
12	8	29	Patients with idiopathic constipation	UBT	IBD: 60, C: 41 CD: NR, UC: NR	Italy	[36]
45	66	77	Patients with non-organic dyspepsia ¹	histology	IBD: 66.7, C: 63.6 CD: 62.2, UC: 69.7	Turkey	[37]
0	90	120	Healthy subjects	Histology, RUT	IBD: 30, C: 52.5 CD: NA, UC: 30	Greece	[38] ²
39	77	127	Healthy subjects ¹	<i>H. pylori</i> IgG (+)	IBD: 31.7, C: 55.1 CD: 28.6, UC: 33.1	Greece	[39] ²
19	21	NA	NA	<i>H. pylori</i> IgG, IgA (+), histology	IBD: 0, C: NA CD: NA, UC: NA	Finland	[40] ³
94	185	70	Healthy subjects ¹	<i>H. pylori</i> IgG, IgA (+)	IBD: 24.4, C: 37.1 CD: 12.9, UC: 29.7	Finland	[41] ²
100	100	100	Patients with acute bacterial diarrhoea ¹	<i>H. pylori</i> IgG, IgA (+)	IBD: 15, C: 43 CD: 13, UC: 18	Finland	[42] ²
147	169	316	Non-IBD patients ¹	UBT	IBD: 25.3, C: 52.5 CD: 17.7, UC: 32	Korea	[43] ²
386	0	277	Blood donors ¹	<i>H. pylori</i> IgG, IgA (+)	IBD: 17.4, C: 35.4 CD: 17.4, UC: NA	Nederland	[44] ²
90	0	525	Non-IBD patients	Histology	IBD: 16.7, C: 40.2 CD: 16.7, UC: NA	Japan	[45] ²
38	0	12	Healthy subjects ¹	UBT	IBD: 8, C: 42 CD: 8, UC: NA	Japan	[46] ²
80	39	98	Non-IBD patients ¹	<i>H. pylori</i> IgG (+)	IBD: 27.5, C: 41.7 CD: 13.5, UC: 30.8	Israel	[47] ²
51	82	200	Non-IBD patients ¹	UBT	IBD: 12.8, C: 39 CD: 13.7, UC: 12.2	Hungary	[48] ²
36	0	36	Healthy subjects ¹	Histology	IBD: 8.3, C: 36.1 CD: 8.3, UC: NA	Germany	[49] ²
75	0	200	Non-CD patients	Histology	IBD: 30.5, C: 35.2 CD: 33, UC: NA	Germany	[50]
56	0	382	Non-CD patients	Histology	IBD: 32.1, C: 46.1 CD: 32.1, UC: NA	USA	[51] ³
371	560	64451	Non-IBD patients	Histology	IBD: 4.5, C: 9 CD: 4, UC: 5	USA	[52] ²
0	42	74	Non-IBD patients	<i>H. pylori</i> IgG (+), UBT	IBD: 52.4, C: 51.4 CD: NA, UC: 52.4	Brazil	[53]
43	0	74	Non-IBD patients	UBT	IBD: 51.2, C: 70.3 CD: 51.2, UC: NA	Brazil	[54]
50	44	194	Non-IBD patients	Histology, RUT	IBD: 9.6, C: 38.5 CD: 14, UC: 4.5	Poland	[55] ^{2,3}
21	23	76	Non-IBD patients	<i>H. pylori</i> IgG (+)	IBD: 54.5, C: 68 CD: 52.2, UC: 57.1	Mexico	[56]
104	104	416	Healthy subjects ¹	UBT	IBD: 19.7, C: 48.8 CD: 18.3, UC: 21.2	Chinese	[57] ²
229	0	248	Non-CD patients	UBT, culture, histology	IBD: 27.1, C: 47.9 CD: 27.1, UC: NA	Chinese	[58] ²
0	153	121	Non-UC patients	UBT, culture, histology	IBD: 30.5, C: 57 CD: NA, UC: 30.5	Chinese	[59]
30	30	20	Non-IBD patients ¹	UBT	IBD: 43, C: 40 CD: 50, UC: 37	Spain	[60]

¹Age and sex matched; ²Statistically significant result (IBD vs control group); ³Paediatric population. CD: Crohn's disease; UC: Ulcerative colitis; C: Controls; IBD: Inflammatory bowel disease; GI: Gastrointestinal; *H. pylori*: *Helicobacter pylori*; Ref: References; NA: Not applicable; NR: Not reported; FAT: Serology fecal antigen test; RUT: Rapid urease test; UBT: Urea breath test.

Table 2 Prevalence of both *Helicobacter pylori* negative and positive gastritis in patients with inflammatory bowel disease in different populations

CD	UC	C	Control group	Biopsies	<i>H. pylori</i> (+) gastritis (%)	<i>H. pylori</i> (-) gastritis (%)	Ref.
37	43	41	Non-IBD patients	Antrum, body	CD: 27, UC: 37.2 C: 53.7	CD: 29.6, UC: 22.2 C: 10.5	[61]
141	79	141	Non-IBD patients	Antrum, angulus, body	CD: 33, UC: 47 C: 60	CD: 43, UC: 12 C: 19	[34]
75	0	200	CD-free patients	Antrum, body	CD: 33.3, UC: NA C: 48	CD: 39, UC: NA C: 0.8	[50]
208	280	4943	Non-IBD patients	Antrum, body	CD: 4, UC: 6 C: 7	CD: 5, UC: 0 C: 0	[63]
67	41	43	Healthy subjects	Antrum, body	CD: 17.6, UC: 6.4 C: 20	CD: 45.4, UC: 15.6 C: 30	[33]
62	0	0	NA	Antrum, corpus	CD: 9.7, UC: NA C: NA	CD: 32, UC: NA C: NA	[64]

CD: Crohn's disease; UC: Ulcerative colitis; C: Controls; IBD: Inflammatory bowel disease; *H. pylori*: *Helicobacter pylori*; NA: Not applicable.

microbiota may be strong confounders, further mechanistic studies in *H. pylori* infection using mouse models are necessary to further define the mechanism of this negative association. Furthermore, when looking for explanations for the lower prevalence of *H. pylori* infection in IBD, some authors have suggested that treatment with sulfasalazine and other aminosalicic compounds could be responsible for “spontaneous eradication” of *H. pylori* infection^[32,34,35,38], although their possible role has not been confirmed by other studies^[29-31,37,39,41-45,55,57,60,99]. Various studies have suggested that sulfasalazine, but not 5-aminosalicylic acid (5-ASA), could account for the lower prevalence of *H. pylori* infection^[32,34], whereas Piodi *et al*^[35] found the opposite. Ishikawa *et al*^[100] observed a lower prevalence of *H. pylori* infection in rheumatoid arthritis patients receiving sulfasalazine, whereas Taha *et al*^[101] did not find any statistically significant difference. The mechanisms of how these agents prevent *H. pylori* infection is still unknown, but prevention may be the result of a direct action against germ adhesion to the gastric mucosa or due to immuno-modulatory actions of the drugs^[30,102,103]. It has also been hypothesized that prolonged treatment with antibiotics used in IBD (especially metronidazole) could account for spontaneous eradication and lower prevalence of *H. pylori* infection. Indeed the prevalence of *H. pylori* infection was significantly lower in CD patients who had received antibiotics for ≥ 2 wk^[45] while in another study, antibiotic therapy was negatively associated with *H. pylori* infection (20.5% *vs* 55%, $P = 0.0001$)^[39]. Moreover, other studies have shown that prior treatment with ciprofloxacin and/or metronidazole had no influence on *H. pylori* status in IBD patients^[48,104,105].

Finally, the data on the prevalence of virulent *H. pylori* strains in IBD patients are limited. Wagtmans *et al*^[44] showed that the majority (66%) of *H. pylori* seropositive patients with CD were infected by *H. pylori* cagA (+) strains although a similar proportion of controls (69.4%) were also infected by these strains. These findings deserve further investigation as it is well known that the intense host responses, specifically to *H. pylori* cagA

(+) strains may further alter Th1- and Th2-type immune responses with subsequent induction of immune-regulatory lymphocytes^[106].

POTENTIAL PROTECTIVE MECHANISMS OF *H. PYLORI* AGAINST IBD

It is plausible to suggest that *H. pylori*, by attempting to promote its own survival, may benefit the host via a variety of mechanisms against other chronic inflammatory conditions such as IBD. Several mechanisms have been proposed to explain the inverse association between *H. pylori* and IBD. In CD, Th1 immune responses prevail, whereas in UC, Th2 or Th1/Th2 immune responses may be predominant^[5,107,108]. These altered immune responses to lumen antigens in IBD may influence the way the host responds to *H. pylori* infection. Conversely, a perpetual bacterial infection in the stomach may either alter the host immune responses in a way that may be protective or render the host susceptible to IBD. The levels of numerous cytokines, including IFN- γ , TNF, IL-1 β , IL-6, IL-7, IL-8, IL-10, and IL-18, are increased in the gastric epithelial cells of *H. pylori* infected humans compared to uninfected humans^[109-111]. After activation of Toll-like receptors by *H. pylori*, dendritic cells (DC) may activate T cells in different ways, being capable of inducing either a Th1 or Th2/regulatory T cell (Treg) response by generation of IL-12 or IL-10, respectively^[112,113]. This finding was reported by D'Elia *et al*^[114] who observed that most (64%) of *H. pylori* specific T cell clones derived from uncomplicated chronic gastritis displayed a Th2-like phenotype, producing interleukin IL-4 or IL-5 together with INF- α , whereas only one third of *H. pylori*-specific gastric T cells were polarized with Th1 effectors.

Thus, a protective role of *H. pylori* infection against IBD may be due to the ability of this microbe to down-regulate pro-inflammatory immune responses. Considering that adoptive transfer of Treg is able to prevent and/or treat colitis in various animal models, it is reasonable to suggest that these cells produced in response to *H.*

pylori infection may act in the prevention of IBD^[115-119]. *H. pylori* can induce a Treg response and down-regulate the pro-inflammatory Th1/Th17 pathway^[65,66,120-123]. The importance of Treg in the pathogenesis of IBD was illustrated by the development of spontaneous colitis in mice deficient of IL-10, a key regulatory cytokine for Treg function^[124].

Moreover, the systemic levels of type I IFN were found to be lower in *H. pylori* infection-colonized IBD patients compared to non-colonized controls^[125]. Luther *et al*^[125] showed that prior oral administration of 20-50 µg *H. pylori* DNA ameliorated the severity of dextran sulphate sodium (DSS) induced acute or chronic colitis in mice in terms of both pathology and symptoms such as bleeding and weight loss. Thus, the protective properties of *H. pylori* DNA were attributed *in vitro* to inhibition of cytokine production by DC, which upon addition of the DNA failed to produce type I interferon and IL-12 in response to *E. coli* DNA^[125]. A protective effect of *H. pylori* colonization in mice against experimental colitis was also demonstrated by Higgins *et al*^[126]. Mice that were colonized with *H. pylori* SS1 6 wk prior to the induction of *Salmonella typhimurium* experimental colitis, experienced markedly less severity inflammation compared to mice that were not colonized with *H. pylori*. This result could be attributed to an up-regulation of IL-10 in the mesenteric lymph nodes and suppression of the Th-17 response in the cecum of the infected mice^[126], illustrating an extra-gastric immune-modulatory effect of the bacterium, an immunological crosstalk between the upper and lower gastrointestinal tract and providing mechanistic support for the epidemiological observation of a negative association between *H. pylori* status and the risk of IBD.

Another protective mechanism may operate via the development of antibodies against *H. pylori*, which may confer an immunization-type protection against other pathogenic *Helicobacter* or even different types of microbes implicated in IBD. Although *H. pylori*-specific antibodies do not eradicate this bacterium, they seem to confer a degree of protective immunity from a subsequent *Campylobacter* infection, indicating an antigenic cross-reactivity between these two bacterial species^[127-129]. It could also be that *H. pylori* induced reduction in acid secretion indirectly affects a different type of infection that ultimately results in IBD. Indeed, variable disease phenotype during dual infection by different *Helicobacter* species has been described by Lemke *et al*^[130] who demonstrated that *H. bilis* and *H. pylori* co-infection in mice attenuates *H. pylori* gastritis compared to those infected only with *H. pylori*.

The protective effect of *H. pylori* may simply be due to other confounding variables such as the presence of inherent genetic or environmental factors that favour *H. pylori* acquisition in some and the development of IBD in others. This scenario would fit well with the observation that IBD is associated with better hygiene, which in itself may be detrimental to *H. pylori* acquisition^[131,132]. The low prevalence of *H. pylori* infection in patients with

IBD compared to non-IBD patients strengthens the importance of the “hygiene hypothesis” in the development of autoimmunity and IBD. It suggests that inadequate microbial stimulation of gut-associated lymphoid tissue is a critical point for maturation of mucosal immunity^[133,134]. Improved access to a cleaner environment and the resulting decreased incidence of common childhood infections, including *H. pylori*, may be contributing to autoimmunity by altering susceptibility to certain diseases with an autoimmune component, such as IBD^[26].

Finally, regarding genetic factors, the CD variant of the autophagy gene ATG16L1 alters susceptibility to *H. pylori* infection with an enteric microbe in human subjects at the population level, supporting a role for altered autophagy in regulating the host response to enteric microbes in CD pathogenesis. It is interesting to speculate that due to increased susceptibility to infection, early exposure and acquisition of *H. pylori* in individuals with the ATG16L risk allele may decrease their risk for the subsequent development of IBD^[135].

ERADICATION OF *H. PYLORI* AND DEVELOPMENT OF IBD

There seems to be a rapid onset of CD after eradication of *H. pylori* infection, as illustrated by two cases^[136]. A similar experience was recently described by Jovanovic *et al*^[137], who described the onset of gastric CD only 6 mo after *H. pylori* infection eradication. Moreover, a steady rise in the incidence of UC was reported in *H. pylori* endemic regions after successful eradication of *H. pylori* infection^[28].

It is unknown why these patients developed CD after eradication of *H. pylori* infection, but this may be due to the induction of immune responses that in turn contributed to the development of the disease. Long-term *H. pylori* infection may cause an unstable equilibrium between the Th1 and Th2 phenotype pattern; eradication of *H. pylori* infection may diminish Th2 cytokine, with sudden consequent Th1 pattern prevalence and rapid increase of pro-inflammatory cytokines^[106]. In genetically predisposed subjects, this Th1 predominant pattern may suddenly favour the onset of a typical Th1-related disease such as CD. Further studies investigating the effect of eradication of *H. pylori* on the development and natural history of IBD are warranted.

CAUSAL ASSOCIATION OF *H. PYLORI* WITH COLORECTAL CANCER?

A meta-analysis of 13 studies suggested an increased risk of colorectal cancer due to *H. pylori* infection^[138]. Kapetanakis *et al*^[139] demonstrated the presence of *H. pylori* in malignant colonic tissue in 34 of 41 (82.9%) patients with colorectal cancer. *H. pylori* colonizing colonic tumour tissue seems to be associated with increased cell proliferation and impaired apoptotic process in malignant tissue

compared with adjacent normal colonic mucosa, thereby further contributing to colon cancer progression^[140]. Furthermore, *H. pylori* induced gastrin release can act as promoter of cell proliferation and differentiation (mainly by inducing COX-2 overexpression and PI3-kinase-mediated tyrosine phosphorylation of E-cadherin and b-catenin) in different gastrointestinal tract sites, including the colon^[141]. *H. pylori* infection is also accompanied by bone-marrow-derived stem cell (CD34+) recruitment that ultimately facilitates colon cancer progression^[142]. Finally, compared to normal gastric mucosa, *H. pylori* gastritis occurred more frequently among patients with hyperplastic polyps (OR = 1.24, 95%CI: 1.18-1.30), adenomatous polyps (OR = 1.52, 95%CI: 1.46-1.57), advanced adenomas (OR = 1.80, 95%CI: 1.69-1.92), villous adenomas or adenomas with high-grade dysplasia (OR = 1.97, 95%CI: 1.82-2.14), and adenocarcinomas (OR = 2.35, 95%CI: 1.98-2.80)^[143]. It has therefore been proposed that *H. pylori* eradication might inhibit IBD-related or non-colon neoplasia^[144].

CONCLUSION

Since the discovery of *H. pylori*, several epidemiological studies, therapeutic trials, case reports and/or *in vitro* studies have been published concerning a hypothetical damaging or protective role of *H. pylori* in the development of IBD. Whether the link between *H. pylori* and IBD is coincidental, epiphenomenal or mechanistic remains uncertain. There are contradictory data regarding both the causative and the protective role of *H. pylori* infection against IBD.

The discordance between studies may be explained by a number of confounding factors, such as variability in the power of the studies and the time periods in which these studies were conducted, geographical factors and the differences in the methods used to detect *H. pylori* infection^[145]. To be more specific, the urease breath test is more sensitive in detecting *H. pylori* than histology. Histology involves the examination of tissue samples that may be insufficient for a correct diagnosis and is more timely than serology, which also detects previous infections. Furthermore, one limitation of the studies using serology for the presence of *H. pylori* is the fact that after successful eradication of *H. pylori* infection, positive titres of antibodies normalize very slowly within several months, or even years, leading to the possibility that negative findings from *H. pylori* serology do not reflect eradication of *H. pylori* infection^[146]. Finally, from a clinical point of view, we must always bear in mind that any type of protection that exerts its influence on a general population level may not necessarily materialize in the individual patient.

In conclusion, the association between *H. pylori* infection and IBD is still controversial; however, it is worthy of further investigation, as the potential association of *H. pylori* with extra-gastric manifestations and disorders is always a very interesting and challenging research area^[147].

It is unclear whether the apparent protective effect of *H. pylori* is simply confounding due to other variables, but the effect of the presence of the live bacterium remains to be elucidated. More studies investigating the effect of *H. pylori* infection eradication on the risk of development of IBD and the natural history of IBD are needed.

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Inflammatory bowel diseases and human reproduction: A comprehensive evidence-based review

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liographies of retrieved articles, books, expert opinion review articles and reviewed bibliographies from subject experts were manually searched. Titles and abstracts were screened initially, and potential relevant articles were identified and reviewed. Whenever possible, data were analyzed by comparing IBD patients *vs* healthy controls, and patients with active IBDs *vs* those with disease in remission. The effects of IBDs on female fertility, fertility in infertile couples, pregnancy and male infertility were examined separately. Patients with IBDs in remission have normal fertility. At the moment, there is no established guideline for the preservation of fertility in women with IBD undergoing surgery. Further data are needed regarding guidelines for the management of these patients. Data regarding IBDs and infertility are currently completely lacking. Considering the prevalence of intestinal pathology in young adults of childbearing age, this field is of great scientific and clinical interest, opening up important future perspectives. Another important and as yet unexplored point is the response to treatments for infertility in patients with IBDs. In particular, the question is whether the reproductive outcomes (clinical and biological) can be influenced by the IBD of one of the partners. The goals for successful reproductive outcomes in IBD population are correct counseling and disease remission. IBDs significantly affect several reproductive aspects of human (female, male, couple) reproduction. Further data are needed to develop guidelines for the clinical management of subjects of reproductive age with IBDs.

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Key words: Inflammatory bowel diseases; Fertility; Infertility; Pregnancy

Core tip: The current comprehensive evidence-based review evaluated the most recent data regarding the

Abstract

To evaluate the effects of inflammatory bowel diseases (IBDs) on human reproduction, we reviewed the current literature using a systematic search for published studies (articles and/or abstracts) without limits for English language. We searched on Medline (through PubMed), the Institute for Scientific Information, the Web of Science and the websites for the registration of controlled trials (<http://controlled-trials.com/>). Bib-

effects of inflammatory bowel diseases on human reproduction.

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INTRODUCTION

Inflammatory bowel diseases (IBDs) predominantly affect younger patients of reproductive age. To date, several reviews^[1-4] have been published in the English literature whose aim was to evaluate and clarify the impact on obstetric outcomes of both the IBDs themselves and the drugs commonly used to treat them. However, very little is known on the overall effect of IBDs on human reproduction.

The study of human reproduction includes not only the effects of IBDs and their treatment on pregnancy but also their effects on the menstrual cycle and hormonal patterns, on the subfertile women scheduled for ovulation induction cycles or assisted reproductive techniques (ARTs), on future reproductive potential in younger and/or adolescent women, and on male fertility, including data on semen parameters and libido/hormonal patterns.

Based on these considerations, the current study was designed to provide an evidence-based overview on the effects of IBDs on all aspects of human reproduction, including both female and male fertility, and to provide a critical synthesis useful for clinical practice.

SEARCH STRATEGY

To obtain evidence-based data and to provide evidence-based recommendations, a systematic search for studies (articles and/or abstracts), without English language limitation, was performed.

It included the combination of the following medical subject headings or keywords: “assisted reproduction techniques”, “ART”, “Crohn’s disease”, “complication”, “effectiveness”, “efficacy”, “embryo”, “endometrium”, “fertility”, “fetal”, “foetal”, “infertility”, “inflammatory bowel disease(s)”, “IBD(s)”, “intracytoplasmic insemination”, “ICSI”, “*in vitro* fertilization”, “IVF”, “libido”, “management”, “menstrual cycle”, “menses”, “neonatal”, “obstetrics”, “ovary”, “pregnancy”, “prevention”, “safety”, “semen analysis”, “sex hormones”, “sperm”, “spermatozoa”, “subfertility”, “surgery”, “therapy”, “treatment”, “UC”, “ulcerative colitis”.

We searched on Medline (through PubMed), the Institute for Scientific Information, the Web of Science, and the websites for the registration of controlled

trials (<http://controlled-trials.com/>). Bibliographies of retrieved articles, books, expert opinion review articles, and reviewed bibliographies from subject experts were manually searched. Titles and abstracts were screened initially, and potentially relevant articles were identified and reviewed.

For each issue, we analyzed mainly meta-analyses and/or randomized controlled trials (RCTs). When meta-analytic data or data from RCTs were lacking, prospective non-randomized and then cohort studies were included in the final analysis.

To construct a comprehensive review, data regarding both primary endpoints, as traditionally suggested by evidence-based medicine (pragmatic view), and intermediate endpoints, crucial to understanding the mechanisms of action (mechanistic view), were extrapolated and analyzed.

Whenever possible, data were analyzed by comparing IBD patients *vs* healthy controls, and patients with active IBDs *vs* those with disease in remission.

FEMALE FERTILITY

Women with inactive Crohn’s disease (CD) or ulcerative colitis (UC) appear to have normal fertility. In remission, female fertility seems not to be diminished. A case-control study by Elbaz *et al*^[5] showed that there was an increased need for fertility treatment of women with IBD; however, this association was no longer significant after controlling for maternal age (increasing maternal age is associated with subfertility).

In CD, fertility is normal or slightly reduced^[6-8]; older referral center studies estimated infertility rates of 32%-42%^[8-11], but community-based and population-based studies suggest infertility rates of 5%-14%, similar to that of the general population^[7]. Women with UC have normal fertility until they undergo surgery^[9,10].

Several reasons for the potentially reduced fertility in IBD women have been hypothesized. We have identified two main sources: psychological problems and surgery-related problems.

Psychological problems

Relatively few data are available regarding sexual dysfunctions in women with IBD. Moody *et al*^[12] did not find any significant change in rates of dyspareunia and overall frequency of sexual intercourse between women with IBD and matched controls. On the other hand, a mismatch of perception and reality seems to significantly affect family planning decisions in women with IBD. A recent large study^[13] was published whose aim was to evaluate whether, and to what extent, IBD patients’ perceptions of risk influence their reproductive behavior and to describe IBD patients’ specific concerns related to fertility and pregnancy. “Voluntary childlessness” was the main cause of the reduced fertility rate (number of live births per woman) reported in IBD patients^[13]. This fear of infertility was most evident in women with CD and pre-

vious surgery^[13]. In particular, IBD-related reproductive risks seemed to be overestimated by the examined subjects. The main reproductive concerns of IBD patients regarded pregnancy risks, drug-related teratogenicity or toxicity, long-term risks and IBD inheritance^[13].

Surgery-related problems

Women with active CD have decreased fertility^[6], perhaps related to the formation of adhesions caused by the disease itself and/or surgery, resulting in tubal infertility^[14]. Fertility may normalize after induction of remission in women with CD^[15]. Some surgical procedures, such as rectal excision and pouch formation, appear to have detrimental effects on male and female fertility.

In UC with ileal pouch anal anastomosis (IPAA), the ability to become pregnant is significantly reduced, probably because of the presence of post-surgical adhesions in the pelvis, secondary obstruction of the fallopian tubes or altering the normal tubo-ovarian relationship necessary for ovum capture and transport^[16-18]. Two studies that evaluated the impact of proctocolectomy with pelvic pouch or end ileostomy on pelvic anatomy, showed that 50 percent of females had complete unilateral or bilateral obstruction of the fallopian tubes^[19,20]. These studies suggested that pelvic dissection is the likely cause of adhesions and altered pelvic anatomy.

A systematic review showed that IPAA for UC results in decreased fertility^[21]. The conception rate in women with UC was 40% before IPAA and only 29% after IPAA^[21]. A meta-analysis estimated that the risk of infertility after IPAA increased by a factor of three^[22]. The high use of fertility treatments after IPAA compared with other groups illustrates the difficulty these women have in getting pregnant after surgery. Olsen *et al*^[10] found that after IPAA, 29% of children were born after *in vitro* fertilization compared with only 1% in the general population. Another study^[10] of Danish and Swedish patients found that females had significantly decreased fecundity (probability of becoming pregnant per month of unprotected intercourse) after IPAA compared with before IPAA. The data regarding fertility after IPAA do not state the number of women who voluntarily chose not to become pregnant and the impact of age on female fertility, which could greatly influence the interpretation of the results.

In a Canadian study, Johnson *et al*^[23] confirmed that the infertility rate was significantly higher in IPAA patients compared with patients managed non-operatively (38.6% *vs* 13.3%). In that study, the effect of age on the risk of becoming pregnant was investigated for all patients who attempted to become pregnant in both the IPAA and non-operative management groups. There was a negative association between advancing age and success of becoming pregnant: for each additional year of age there was a 12% decrease in the odds of becoming pregnant^[23]. Increasing age was associated with decreased reproductive ability among all females included in that study^[23].

A recent Finnish study showed that, although the probability of a women conceiving in any short time period seemed to be reduced to 47% of the average, the lifetime chance of having at least one live birth after IPAA was 80%^[24]. Thus, women with IPAA mostly suffer a reduction in the probability of conception rather than complete infertility.

Strategies are needed to improve fertility after IPAA. One possibility would be to perform sub-total colectomy/end ileostomy and delay IPAA, but this would likely be unacceptable to most females. A second strategy would be to preserve tubal patency and normal tubo-ovarian relationship. Various materials are available to prevent post-surgical adhesions in the pelvis; however, as there are no data regarding their efficacy in improving fertility, further research is needed^[25].

To date, there are no established guidelines for the preservation of fertility in women with IBD undergoing surgery. However, we believe that an effective strategy should be based on the following principles: (1) proper selection of patients with a specific clinical indication for surgery; (2) proper evaluation of the patient based on factors predictive of ovarian reserve [*i.e.*, age, anti-Müllerian hormone (AMH), antral follicle count]; and (3) surgery that is as minimally destructive of the radical pelvic anatomy as possible.

FERTILITY IN INFERTILE COUPLES

The purpose of this section is to evaluate the effects of the IBDs in the infertile couple. In particular, our attention focused on reproductive outcomes (*i.e.*, all intermediate steps and final results) of an infertile couple in which one or both partners are affected by IBDs. Both CD and UC mainly affect young adults of reproductive age. Whereas substantial data are available concerning pregnancy in young IBD women or fertility in IBD men, no study has been conducted on IBDs in infertile couples.

In the few last years, only two studies^[26,27] investigated the ovarian reserve status in CD women, as reflected by serum AMH. The first study^[26] showed that women with CD do not have severe ovarian reserve alterations compared with a control population. However, age \geq 30 years and a colonic location of the disease could be associated with an accelerated loss of follicles. Another study^[27] confirmed that serum AMH levels of reproductive-age women with CD were significantly lower compared with the controls, and the Crohn's Disease Activity Index (CDAI) and AMH were inversely correlated. Thus, these data could encourage gastroenterologists to inform CD women of the risk of delaying childbirth.

Finally, only one report^[28] described the reproductive outcome following intracytoplasmic sperm injection (ICSI) for male factor infertility associated with CD and 6-mercaptopurine (6-MP) chemotherapy. The authors^[28] reported the first successful birth after ICSI for severe oligozoospermia associated with CD.

PREGNANCY

Several recent data are available in literature on IBDs and pregnancy. However, the majority of published studies are reviews or retrospective analyses.

Effects of pregnancy on IBDs disease activity

Pregnancy seems to have a beneficial effect on IBD symptoms, especially when it occurs during disease remission. A small, but significant, decrease in the Harvey-Bradshaw index of disease activity during pregnancy, in comparison with the year preceding and following pregnancy, was observed in a retrospective analysis on women with CD^[29]. Similar results were found in a large European prospective study, showing that 74% of CD and 67% of UC patients with active disease at conception achieved remission later during pregnancy^[30].

Factors affecting remission or exacerbation of IBDs have been investigated extensively. In particular, smoking has a negative effect on the course of CD, and some authors showed that the reduced disease activity in pregnancy was partly the result of reduced tobacco smoking during pregnancy^[31]. By contrast, exacerbation of disease, particularly in the first trimester of pregnancy, could result from discontinuation of maintenance therapy.

The state of the disease at conception is a factor influencing the course of pregnancy^[32-34]. In fact, patients with active disease at conception often continue to have symptoms during pregnancy, whereas a normal course of pregnancy can be expected in patients who conceive when in remission. In a cohort study with a 10-year follow-up period, it was observed that if conception occurred during remission, the risk of a flare-up was comparable to that in non-pregnant patients with IBD. Instead, when conception occurred during an active disease period, two-thirds of patients relapsed during pregnancy and more than 60% of these patients experienced further deterioration^[35].

The course of IBD in the postpartum period remains controversial. A small prospective study reported a decrease in relapse rate in CD, as well as in UC patients, 4 years after pregnancy, in comparison with the 3 years before pregnancy^[36]. Similarly, in a large cohort study, the yearly flare-up rates decreased from 0.34 to 0.18 in UC and from 0.76 to 0.12 in CD. Two studies^[37,38] also reported reduced stenosis and resection rates in women with IBD after pregnancy. Mechanisms potentially involved could be related to the hormone relaxin, the effect of pregnancy on the immune response, as well as feto-maternal HLA disparity^[38,39]. The effects of breastfeeding on flare-up rates in IBD mothers are also controversial^[40,41].

Effect of IBDs on pregnancy/perinatal outcomes

Current data indicate that quiescent disease has minimal impact on the course and outcome of pregnancy in IBD patients, whereas patients with active disease at conception have increased rates of spontaneous abortion^[33] and a significantly increased risk of preterm delivery and low

birth weight^[32,42]. Overall pregnancy outcomes in women with IBDs (CD or UC) were similar to those of non-IBD pregnant patients^[31].

A meta-analysis^[43], including 3907 subjects, reported increased risks for preterm delivery (OR = 1.87, 95%CI: 1.52-2.31) in both CD and UC patients; low birth weight (OR = 2.82, 95%CI: 1.42-5.60) in CD but not in UC; and congenital abnormalities (OR = 3.88, 95%CI: 1.14-10.67) in UC but not in CD. A Swedish population study found 4.5% and 1.2% of children born to IBD patients had, respectively, low and very low birth weight, as compared to 2.9% and 0.6% in the overall Swedish population^[44]. Moser *et al.*^[32] additionally found an increased incidence of poor maternal weight gain during pregnancy in CD patients with quiescent disease at conception.

In both CD and UC patients, pregnancies ended more frequently with caesarean section compared with the general population^[45]. Furthermore, pregnant CD and UC patients who needed to be hospitalized had a higher risk of undergoing a caesarean section (OR = 1.72, 95%CI: 1.44-2.04 and OR = 1.29, 95%CI: 1.01-1.66, respectively) in comparison with patients without IBD^[46].

Prevention and treatment of IBDs during pregnancy

Most of the drugs used to treat IBD are not associated with increased risk of congenital anomalies or adverse effects on the fetus (Table 1). The 2010 European Crohn's and Colitis Organisation (ECCO) guidelines state "medical treatment for Crohn's disease (except methotrexate) should generally continue during pregnancy, because the benefits outweigh the risks of medication"^[46]. Moreover, as complications and adverse pregnancy outcomes mainly occur in patients with active disease, the main concern should be to achieve remission before conception and maintain quiescent disease during pregnancy. Patients who received counseling regarding the benefits and risks of drug treatment before conception and during pregnancy were more likely to remain compliant^[34].

It is generally regarded as safe to keep using aminosalicylates (ASA) during pregnancy [Food and Drugs Administration (FDA) category B drug], despite some reports noting a higher incidence of neural tube defects, oral cleft and cardiovascular defects^[47]. In a recent meta-analysis of treatment of IBD patients with 5-ASA drugs, IBD did not significantly increase the risk of congenital abnormalities (OR = 1.16), stillbirth (OR = 2.38), spontaneous abortion (OR = 1.14), preterm delivery (OR = 1.35) or low birth weight (OR = 0.93)^[48].

Several studies^[49,50] reported that sulfasalazine assumption during pregnancy does not give rise to increased rates of birth defects in women with IBD. Sulfasalazine therapy should be accompanied by extra folate supplementation, as this medication halts folate synthesis by inhibiting dihydrofolate reductase. Folic acid supplementation decreases the augmented risk of oral clefts and cardiovascular anomalies associated with folate antagonist treatment during pregnancy^[51]. Caution should be applied regarding the use of some mesalamine formulations (*e.g.*,

Table 1 Safety indications for drugs in pregnancy and breastfeeding

	UC	ECCO rating pregnancy	ECCO rating breastfeeding	Personal observations
5-ASA (except sulfasalazine)	B	Safe	Safe	Avoid high doses for long time, asacol preparations may be switched to another mesalamine
Sulfasalazine	B	Safe	Safe	Folate supplements are required
AZA/6-MP	D	Safe	Probably safe	Discuss breastfeeding with the patient. Avoid lactation in the four hours after intake of the drug
Cyclosporine A	C	Probably safe	Contraindicated	Use only in severe cases of UC to avoid urgent colectomy during pregnancy
Methotrexate	X	Contraindicated	Contraindicated	Discontinue at least 4 mo prior to conception
Corticosteroids	C	Safe	Safe	Very low risk of malformations. If possible, not use for long time
Infliximab	B	Probably safe	Safe	Can be used safely in the first two trimesters of pregnancy. If possible, avoid in the third trimester
Adalimumab	B	Probably safe	No data	Can be used safely in the first two trimesters of pregnancy. If possible, avoid in the third trimester
Metronidazole	B	Probably safe	Best avoided	Very slight increase of cleft lip. Use only if strictly necessary
Ciprofloxacin	C	Probably safe	Probably safe	Use only for short periods and avoid in the first trimester
Thalidomide	X	Contraindicated	Contraindicated	

5-ASA: 5-aminosalicylates; AZA: Azathioprine; 6-MP: 6-mercaptopurine; ECCO: European Crohn's Colitis Organisation; UC: Ulcerative colitis.

asacol) that contain dibutyl phthalate (DBP) as a coating agent. The use of DBP-coated medications produces measurable phthalate metabolite levels in urine. Prenatal exposure to DBP can cause congenital malformations in the male urogenital tract^[52]. Finally, the sulfasalazine metabolite sulfapyridine is secreted into breast milk. ASA are generally considered safe during lactation, although a case of bloody diarrhea in an infant has been reported^[53-55].

The use of antibiotics during pregnancy, *i.e.*, matronidazole and quinolones, should be considered with caution. Metronidazole is considered a low-risk drug during pregnancy (FDA class B). While several studies did not find an association between metronidazole treatment and birth defects^[56], a large case-control study showed an increased incidence of cleft lip and/or cleft palate in infants of mothers exposed to metronidazole in the first trimester of pregnancy^[57]. Thus, it should be limited to short-term use for the treatment of pouchitis. In addition, as metronidazole is excreted in breast milk, breastfeeding during its administration is not recommended^[56].

Quinolone antibiotics are FDA category C drugs and should be avoided because they carry an increased risk of arthropathy because of their high affinity for bone and cartilage. ECCO recommends avoiding quinolone use in the first trimester of pregnancy^[57]. Data on breastfeeding are limited, but quinolone use is probably compatible with breastfeeding^[58,59].

The recent London position statement on biological therapy for IBD states that anti-tumor necrosis factor (TNF) therapy is considered low risk and can be used in the preconception period and during the first two trimesters of pregnancy^[60]. The cytokine TNF- α not only plays a pivotal role in the inflammation process underlying IBD, but also plays physiological roles in host defense mechanisms and pregnancy. During pregnancy, TNF- α probably plays a role in protecting the fetus against teratogenic stress^[61]. Despite the role of TNF in pregnancy, treatment with anti-TNF antibodies can be considered

safe in the preconception period and the first part of pregnancy, because IgG antibodies do not cross the placenta in the first pregnancy trimester and transplacental IgG transport mainly takes place during the late second and third trimester of pregnancy^[62,63]. Maternal transfer of IgG during the last trimester of pregnancy provides the neonate with sufficient acquired immunity to defend itself while its own immune system is becoming fully functional.

The currently available TNF inhibitors (etanercept, infliximab, adalimumab, golimumab and certolizumab) are all classified as FDA category B drugs, indicating that no teratogenic effects of these drugs were observed in animal reproduction studies; however, adequate and controlled human safety data are still lacking. Transfer of anti-TNF antibodies to the fetus during the last part of pregnancy may mean exposure of the neonate in the first months after birth, raising potential concerns about infection and response to vaccines^[60].

Infants exposed to immunosuppressive drugs during pregnancy probably should be considered to be immunocompromised, as are their mothers. ECCO guidelines state that live vaccinations (BCG, rotavirus, mumps-measles-rubella (MMR) and varicella zoster) are contraindicated until exposure to immunosuppressants has been discontinued for at least 3 mo^[64]. IgA is the predominant immunoglobulin in human milk, so secretion of TNF inhibitors in milk is likely to be very limited and breastfeeding under anti-TNF treatment can be considered safe^[65].

Natalizumab is an α -4 integrin inhibitor approved for treatment of CD in the US, but not in Europe. Experience with natalizumab in the context of IBD is still limited, even if this biological compound is widely used for treating multiple sclerosis. It has received an FDA category C label. Thus, at present insufficient data are available to reach a definite conclusion on the safety of natalizumab during pregnancy and lactation.

Corticosteroids are FDA category C drugs. They are

believed to be safe throughout pregnancy at doses up to 15 mg per day^[60], whereas higher doses increase the risk of infection and premature delivery^[66]. Systemic treatment with corticosteroids during the first trimester of pregnancy was found to slightly increase the risk of oral clefts (OR = 3.35, 95%CI: 1.97-5.69), while the overall risk of congenital malformations is not significantly increased (OR = 1.45, 95%CI: 0.80-2.60)^[67].

Pregnant women are preferably treated with prednisone or prednisolone, as the bulk of these compounds are inactivated by placental 11 β -hydroxy steroid dehydrogenase, the physiological mechanism in place to protect the fetus from elevated maternal cortisol levels during pregnancy. Treatment with corticosteroids is compatible with breastfeeding^[68,69].

Cyclosporine crosses the placenta but is rapidly cleared in the neonate and has no known teratogenic effect. FDA categorizes cyclosporine in pregnancy category C for lack of controlled studies in humans; however, cyclosporine does not appear to be a major teratogen.

A meta-analysis^[58] on the use of cyclosporine in pregnancy showed that cyclosporine use in pregnancy was not associated with major malformations, but slightly decreased birth weight and duration of gestation. In IBD patients, cyclosporine use in refractory UC during pregnancy has been shown to be safe and effective. Its main use in pregnant IBD patients is the prevention of urgent colectomy in fulminant UC^[70,71].

Although a number of cases are reported where no overt adverse effects were observed in breastfed infants of mothers treated with cyclosporine, the use of this drug during lactation is generally not advised as cyclosporine is secreted in milk at high concentrations, leading to potential nephrotoxicity and immunosuppression in exposed infants^[72].

ASA and 6-MP are still designated as FDA category D drugs, indicating that increased risk for the fetus exists, but the risk must be weighed against the possible benefits of the drug. A recent Danish cohort study showed an increased risk of preterm delivery and low birth weight in women exposed to azathioprine (AZA) or 6-MP during pregnancy, but no significant increase in congenital malformations^[50].

The CESAME study^[73], a cohort study comparing IBD patients exposed to thiopurine therapy during pregnancy with women receiving other treatments or women without any drug therapy, showed that thiopurine exposure was not associated with low birth weight prematurity and congenital abnormalities, but was associated with preterm birth. Exposure in men at the time of conception was not associated with congenital abnormalities^[74]. Thiopurine treatment is generally considered a contraindication for breastfeeding.

Methotrexate has teratogenic properties and is contraindicated during pregnancy (FDA category X). Methotrexate metabolites have long tissue half-lives; therefore, its administration must be stopped 3 to 6 mo before conception^[75]. Folic acid supplementation after methotrexate

withdrawal is also recommended because methotrexate acts as a folate antagonist. Methotrexate is also contraindicated during lactation because of its potential accumulation in the child's tissues.

Thalidomide and its analog lenalidomide partly counteract the effects of TNF- α and have been used in patients with refractory CD, even if currently available systematic evidence does not clearly demonstrate the benefit of these drugs^[76]. The teratogenicity of these drugs has been well documented, indicating increased risks for limb defects, central nervous system defects, and congenital abnormalities in the cardiovascular, respiratory, gastrointestinal, and genitourinary tracts. Thus, thalidomide is absolutely contraindicated in pregnancy (FDA category X) and patients taking thalidomide are advised to use two complementary contraceptive methods^[77,78]. Although lenalidomide appears less teratogenic in animal studies, the lack of studies demonstrating its safety in humans leads to the absolute contraindication of this drug in pregnant patients or in patients wishing to become pregnant^[77,78].

Management of delivery in women with IBDs

Pregnancy should not be considered high risk in all cases of IBDs. Delivery, therefore, can be carried out in clinics of the second or third level. Only patients with active IBD necessitating steroid and/or anti-TNF treatment, patients with ileostomies, and patients with ileoanal pouches should be referred to high-risk pregnancy clinics.

Active perianal disease at the time of delivery is an indication for caesarean section, whereas patients without history of perianal disease or inactive perianal disease do not require caesarean delivery^[45]. In UC patients with IPAA, caesarean section rates of almost 50% have been reported, but the incidence of pouch-related complications was low and pouch function was found to be unrelated to the mode of delivery^[79]. However, the most recent ECCO guidelines state that the presence of an ileoanal pouch in CD patients is an indication for caesarean section^[80]. Moreover, patients with IPAA surgery in the past are always advised to have caesarean section, and we recommend that an abdominal surgeon should be present during the surgery.

MALE FERTILITY

Clinical data

Infertility in men with IBD has been relatively less studied than infertility in women with IBD. A recent European consensus states that both male and female fertility are not significantly affected in non-operated IBD patients when disease is quiescent, compared to the general population^[81]. It is important to note that IBD patients remain voluntarily childless more frequently than non-IBD controls^[82]. In a survey among 255 Australian IBD patients, fear concerning IBD heritability, side effects of the medication on the child, and medical advice given by physicians were the most important reasons for voluntary childlessness^[13].

Table 2 Effects of inflammatory bowel disease medications on erectile dysfunction, male infertility and partner's pregnancy

	Erectile dysfunction	Infertility	Pregnancy complications	Recommendations
5-ASA (except sulfasalazine)	No	Single reversible case	No reports	No recommendations to discontinue prior to conception
Sulfasalazine	Single case	Yes, reversible, not dose dependent	One study	Switch to other 5-ASA preparations
AZA/6-MP	No reports	No	Controversial	No recommendations to discontinue prior to conception
Cyclosporine A	No reports	No	No reports	No recommendations to discontinue prior to conception
Methotrexate	Yes	Controversial	No reports	Discontinue 3-4 mo prior to conception
Steroids	No reports	No	No reports	Lack of data to discontinue
TNF- α inhibitors (infliximab, and only a case report for adalimumab)	No reports	Reduce sperm quality, but no infertility	No	No recommendations to discontinue prior to conception, but barrier methods during pregnancy

5-ASA: 5-aminosalicylates; AZA: Azathioprine; 6-MP: 6-mercaptopurine; TNF: Tumor necrosis factor.

A recent systematic review^[83] on fertility in non-surgically treated IBD showed that in men with CD (a total of 493 men in two population-based and one referral center studies), there was an 18%-50% reduction in fertility compared with controls, although whether the cause was involuntary infertility or voluntary childlessness was not indicated. There was no evidence of reduced fertility in men with UC^[83].

IBD and male fertility intermediate end-points

Active disease, IBD treatment, and psychological factors affect male reproductive and sexual function^[84]. Most male IBD patients considered "maintaining remission" as important at conception^[85] and in fact inflammation has a negative effect on male fertility^[86]. Men who were in remission or who had only mild disease activity had rates of erectile dysfunction similar to those of healthy control subjects, whereas men with more severe IBD activity had higher rates^[87].

IBD and semen parameters

There are no large studies that assess semen abnormalities in IBD patients on no medication at all. Some studies suggest that factors such as disease activity and nutritional status could affect semen^[88-90]. Furthermore, there are reports of antisperm antibodies in both men and women with IBD, and these antibodies might contribute to infertility. Antisperm antibodies might be a result of the increased immunological response, caused by increased intestinal permeability, against antigens of gut microbiota possessing common antigenicity with spermatozoa^[91]. Dimitrova *et al*^[92] showed that antisperm antibody incidence in 50 patients with ulcerative colitis was statistically significant compared with 50 healthy blood donors^[92]. A little study on 10 IBD patients (four women and six men) and reported the presence of antisperm antibodies in semen of male patients and cervical secretions of female patients at the time of ovulation^[93].

Surgery and male fertility

The most common surgery in IBD patients is IPAA.

Proctocolectomy with IPAA has been associated with sexual dysfunction in men. A large study found 1% and 2% of sexual dysfunction at 1 year and 12 years after surgery, respectively^[94]. Furthermore, 3% of men reported experiencing either retrograde ejaculation or no ejaculation 10 years after surgery^[94].

A meta-analysis of 43 observational studies that evaluated patients after IPAA found the pooled incidence of sexual dysfunction to be 3.6%^[95]. On the other hand, a study of 122 men who had undergone IPAA evaluated male sexual function using a validated index based on erectile function, orgasmic function, sexual desire, intercourse satisfaction and overall satisfaction^[96]. This study showed that, despite any negative effect on erectile function (in particular retrograde ejaculation), the other four features had a statistically significant improvement^[96]. A Dutch study confirmed that despite an elevated rate of sexual dysfunction of up to 25% in 35 men after IPAA for UC, 90% of patients were satisfied with the operation^[97].

A randomized, placebo-controlled trial of sildenafil for erectile dysfunction following rectal excision performed either for cancer or IBD in 32 men showed that post-operative sexual dysfunction can be treated successfully with sildenafil in most cases (79% of the sildenafil-treated group compared to only 17% of the group taking placebo)^[98].

There are other types of surgery beyond IPAA, for example, total colectomy with end ileostomy, colectomy with ileo-rectal anastomosis, and proctocolectomy with ileo-anal anastomosis without a pouch. The reports on sexual function after these procedures are limited, but only ileo-rectal anastomosis avoids the extensive pelvic dissection of the other procedures, which produces adverse effects on sexual function.

Medical treatments and male fertility

Table 2 shows the effects of IBD medications on erectile dysfunction, infertility and pregnancy. In the general population, medications are responsible for erectile dysfunction in up to 25% of cases^[99]. The medications used

to treat IBD rarely cause erectile dysfunction; however, antidepressants and anti-anxiety medications, frequently prescribed for patients with IBD, could cause erectile dysfunction because depressive mood negatively influences sexual function (patients with a first diagnosis of IBD had higher rates of depression than patients with diagnosis of colon cancer)^[100].

On the other hand, many drugs have been reported to impair semen parameters; sulfasalazine, methotrexate, and infliximab seem to affect sperm quality^[57]. Of note, confounding factors that could affect male fertility, such as smoking, alcohol consumption, disease status, and medication use, are not always reported in studies investigating the effects of medical treatments on male fertility. Men with IBD rarely have documented physician counseling regarding the potential effects of medication on fertility and pregnancy because gastroenterologists' lack of knowledge of the effects of medications on male fertility or a lack of documentation of their counselling practices^[101].

Except for sulfasalazine, the risk of adverse fetal outcomes of other medications must be weighed against the benefit of maintaining good health of the father at the time of conception.

The only 5-ASA medication shown to cause male infertility is sulfasalazine^[102,103]. Sulfasalazine causes reversible non-dose-dependent semen abnormalities (oligospermia, reduced motility and abnormal morphology)^[104,105] and infertility in up to 60% of men^[106]. Impaired sperm maturation and oxidative stress from the sulfapyridine constituent of the drug are thought to be the cause^[107-110]. Male fertility is restored two months after sulfasalazine withdrawal or switching to other mesalazine preparations^[111-115]. A single case of impotence was reported that was resolved after switching to olsalazine^[116]. One study found an association between sulfasalazine use in men and a higher rate of congenital malformations in their children^[106]. There is a single case report of mesalazine-induced oligospermia and infertility that reversed when the drug was stopped^[117]. Given these data, no recommendations are made to discontinue 5-ASA (except sulfasalazine) for men planning to conceive.

A study of 18 men with IBD showed that AZA did not reduce semen quality, and thus male fertility, in IBD: no changes in semen parameters were noted after 11 ± 5 mo of AZA administration or during long-term treatment (49 ± 14 mo)^[118]. In a survey of 164 male renal transplant patients, long-term therapy with cyclosporine, AZA and prednisone demonstrated no effects on fertility^[119].

The teratogenic effect of AZA and its metabolite 6-MP remains controversial. In one retrospective study, the incidence of pregnancy-related complications was significantly increased when the fathers used 6-MP within 3 mo of conception^[120]. Specifically, in men with IBD under 6-MP, congenital anomalies and spontaneous abortions were detected in 4% and 6% of cases, respectively. These data are substantially different from those ob-

served in men with IBD who were not taking this drug (no congenital anomalies and 2% of spontaneous abortions)^[120]. However, the rates of congenital abnormalities and spontaneous abortions were below those of the general population - 3% and 10%, respectively^[121]. In a Danish population-based cohort study, the paternal use of AZA or 6-MP before conception was associated with an increased, but not statistically significant, risk of congenital abnormalities^[122].

Two other studies did not observe any significant effect of preconception thiopurine exposure of the father on pregnancy outcomes. Francella *et al.*^[123] showed that there was no statistical difference in conception failures (defined as a spontaneous abortion), abortion secondary to a birth defect, major congenital malformations, neoplasia or increased infections among offspring of male patients taking 6-MP compared with controls. In 2010, Teruel *et al.*^[124] confirmed there were no significant differences in terms of unsuccessful pregnancies between the exposed group (MP 9, AZA 37) and the control group. Given these data, no recommendations are made to discontinue AZA/6-MP for men planning to conceive.

There are no data regarding the effects of cyclosporine on fertility of men with IBD. In animal models, cyclosporine A causes damage of testicular tissue and sperms^[125], but antioxidants may protect from testicular toxicity^[126,127]. In humans, the small studies conducted do not seem to suggest any association between cyclosporine and male infertility: a decrease in serum antisperm antibodies^[128,129], normal semen analysis, and successful pregnancies are described^[130]. There are no reports of adverse pregnancy among partners of men who were taking cyclosporine. No recommendations are made to discontinue cyclosporine A for men planning to conceive.

Methotrexate is the one most often associated with impotence^[131-133]. However, few data are available on the effects of methotrexate on male reproductive capability. In animal models, it causes altered spermatogenesis and degeneration of spermatocytes, Sertoli cells, and Leydig cells^[134-136]. Opinions differ in the literature on the effects of methotrexate on male fertility: the concurrent administration of other chemotherapeutic agents is a limitation^[137].

There are small studies reporting on methotrexate use with no other agents. In patients affected by psoriasis, there are cases of documented reversible sterility when the methotrexate was stopped^[138] and there are several case series that report no change in sperm quality^[139-142]. There are no reports of adverse pregnancy outcomes among the partners of men exposed to methotrexate before conception^[143]. However, the active metabolites of methotrexate can remain in cells or tissues for several months after discontinuation^[144]. Given these data, it is recommended that the drug should be discontinued at least 3-4 mo before attempts at conception for men with IBD^[145-147].

Few data are available on the effects of steroid therapy on male fertility^[118,148]: it appears there is little

impact. Although steroids may inhibit apoptosis of damaged sperm cells, they may prevent pathological excessive apoptosis, which results in oligospermia^[149]. No recommendations can be made to discontinue steroid use due to insufficient data.

There are few studies also on the effects of anti-tumor necrosis factor agents; the data do suggest, however, that these medications may impair male fertility. Infliximab may also serve to counter the negative effects of TNF- α on sperm quality^[150]: supraphysiological levels of TNF- α were seen to cause chromatin and DNA damage, and reduce sperm motility^[151]. Reports on male fertility in ankylosing spondylitis patients showed a decrease in sperm motility in patients on conventional treatment *vs* anti-TNF- α -treated patients^[152].

In a study of 10 men, infliximab increased semen volume with a trend towards decreased sperm motility and morphology, but its impact on male fertility was not reported^[153]. Villinger *et al*^[154] showed sperm abnormalities that were more pronounced in patients with active spondyloarthritis, but the sperm quality of patients with inactive disease receiving long-term TNF inhibition is comparable to that in healthy controls. Another report on four patients with ankylosing spondylitis who fathered six healthy children during infliximab treatment may provide some reassurance for male patients treated with infliximab^[155]. The data support continuation of anti-TNF treatment when IBD patients plan fatherhood^[154].

The recent Austrian evidence-based consensus on the safe use of infliximab in inflammatory bowel disease^[156] states that infliximab may lead to reduced quality by decreasing sperm motility and affecting sperm morphology, although these findings do not demonstrate that male fertility would be reduced^[157].

Infliximab treatment of men prior to planned conception does not seem to cause embryo toxicity. For anti-TNF alpha, as it is unknown whether the fetus will be affected by exposure to anti-TNF alpha through semen, the ECCO consensus on reproduction in IBD advises barrier methods during pregnancy. Data on the impact of other biologicals on male fertility are currently lacking, although there is a case report of 35-year-old father of a healthy 4-year-old child who was successfully treated for 3 years with adalimumab for ankylosing spondylitis^[158]. No recommendations are made to discontinue anti-TNF- α treatment for men planning to conceive.

CONCLUSION

IBDs significantly affect several reproductive aspects of human (female, male, couple) reproduction. Moreover, further data are needed in order to develop guidelines for the clinical management of subjects of reproductive age with IBDs. In fact, at the moment, there are no established guidelines for the preservation of fertility in women with IBD undergoing surgery. Further data are needed regarding the management of these patients.

Data regarding IBDs and infertility are currently com-

pletely lacking. Considering the prevalence of intestinal pathology in young adults of childbearing age, this field is of great scientific and clinical interest, opening up important future perspectives. Further studies should, in fact, be conducted to determine whether the treatments for infertility have an effect on the state of IBD (remission, flare-up, recurrence). Another important point as yet unexplored is the response to treatments for infertility in patients with IBDs. In particular, the question is whether the reproductive outcomes (clinical and biological) can be influenced by the IBD of one of the partners.

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***Mycobacterium avium* subspecies *paratuberculosis* causes Crohn's disease in some inflammatory bowel disease patients**

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are many. Examples include its extreme slow growth, lack of cell wall, low abundance, and its mycobactin dependency. In this review article, data from 60 studies showing the detection and isolation of MAP by PCR and culture techniques have been reviewed. Although this review may not be 100% comprehensive of all studies, clearly the majority of the studies overwhelmingly and definitively support the role of MAP in at least 30%-50% of CD patients. It is very possible that lack of detection of MAP from some CD patients may be due to the absence of MAP role in these patients. The latter statement is conditional on utilization of methodology appropriate for detection of human MAP strains. Ultimately, stratification of CD and inflammatory bowel disease patients for the presence or absence of MAP is necessary for appropriate and effective treatment which may lead to a cure.

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Abstract

Crohn's disease (CD) is a chronic inflammatory condition that plagues millions all over the world. This debilitating bowel disease can start in early childhood and continue into late adulthood. Signs and symptoms are usually many and multiple tests are often required for the diagnosis and confirmation of this disease. However, little is still understood about the cause(s) of CD. As a result, several theories have been proposed over the years. One theory in particular is that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is intimately linked to the etiology of CD. This fastidious bacterium also known to cause Johne's disease in cattle has infected the intestines of animals for years. It is believed that due to the thick, waxy cell wall of MAP it is able to survive the process of pasteurization as well as chemical processes seen in irrigation purification systems. Subsequently meat, dairy products and water serve as key vehicles in the transmission of MAP infection to humans (from farm to fork) who have a genetic predisposition, thus leading to the development of CD. The challenges faced in culturing this bacterium from CD

Key words: *Mycobacterium paratuberculosis*; Crohn's disease; Culture; PCR; Johne's disease; Inflammatory bowel disease

Core tip: The review manuscript describes the past, present and predicted future research accomplishments in the area of Crohn's disease and *Mycobacterium avium* subspecies *paratuberculosis*. This is a highly debated area and Dr. Naser's thoughts described in this review will fuel interest and discussions in inflammatory bowel disease research. The manuscript has been in preparation for a couple of years and it is of high quality.

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INTRODUCTION

One of the earliest documented descriptions of Crohn's disease (CD) was described in 1769 by Giovanni Battista, an Italian physician. He described the results of an autopsy of a man who had suffered from chronic bowel movements throughout his life and subsequently died from diarrhea and fever^[1]. This may have been the first account of granulomatous inflammatory bowel disease (IBD). Several years later in 1813, Combe and Saunders observed a patient who suffered from an abnormally narrow and thickened ileum^[1] and Abercrombie had reported a case in 1828 whereby a patient suffered from an inflamed ileum (ileitis) as well as skip lesions affecting certain segments of the ascending colon and cecum^[1]. There were several medical publications made in the 20th century which provided further insight into the characteristics and features of CD. For example, some such cases were reported by Braun (1901), Koch (1903), Lesniowski (1903), Wilmanns (1905), Moynihan (1907), and Proust (1907)^[1]. In 1913 a surgeon by the name of Dalziel (1861-1924) had reported the symptoms of several of his patients that closely resembled clinical manifestations in cattle suffering from Johne's disease^[2]. He is credited as being the first scientist to hypothesize that the causative agent of Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis* (MAP), may in fact be responsible for chronic intestinal inflammation observed in the intestines of humans. In 1923 Moschcowitz and Wilensky^[3] had reported four cases of young patients suffering from non-specific granulomata of the intestine. In these patients they observed what appeared to be tumor-like masses that were hard, thick, and associated with all four coats of the large intestine which caused stricture of the lumen. Based on these observations it was originally believed that these structural changes were confined to the colon. However, it was found that such lesions could also be located in the small intestine which was later seen in one patient^[4]. In fact, it is interesting to note that all four of the patients had a history of appendicitis and appendectomy.

In 1932 acknowledgement of CD as an official medical entity was as a result of an article published by Drs. Burrill Crohn, Leon Ginzburg, and Gordon G. Oppenheimer, who all worked at Mount Sinai Hospital in New York at the time. Their article entitled "Regional ileitis: A Pathological and Clinical Entity" had appeared in the *Journal of the American Medical Association* in October 1932^[5]. The title "Crohn's disease," has been coined after Dr. Burrill B. Crohn, a gastroenterologist who presented the above named paper at the annual American Medical Association in May 1932. The study described a disease that exclusively affected the terminal ileum of 14 patients from a pathological and clinical standpoint^[5]. The patients were primarily young adults of ages ranging from 17 to 52 years, but only two of them were older than 40 years^[5]. It was expressed that this was a moderately acute disease that was associated with inflammation characterized by rapid necrosis throughout the affected tissue, and by inflammation associated with scar tissue^[5]. Furthermore, it

was indicated that the disease is clinically represented by certain common symptoms similar to ulcerative colitis—fever, diarrhea, and even weight loss^[5]. Dr. Crohn *et al*^[5] also witnessed that the ulcers associated with the mucosa were accompanied by a non-uniform connective tissue reaction of the remaining walls of the involved intestine, a process which frequently leads to the narrowing of the lumen of the intestine, and this has been known to be associated with the formation of multiple fistulas. Other physicians reported of similar concurrent observations, but these reports cited the involvement of a number of different parts of the gastrointestinal (GI) tract. For instance, the first case reporting evidence of inflammation present in the colon and not just in the ileum was by Colp in 1934^[6]. His report is considered as the first case detailing of ileocolitis which described that this inflammatory process could also extend to the cecum and the ascending colon^[6]. In addition, several years later granulomatous lesions were also found in the skin^[7]. As a result, it was becoming quickly apparent that CD is a chronic inflammatory disease that can affect any region of the GI tract ranging from the mouth to the anus, the ileum being the most commonly targeted site. In 1938, Penner and Crohn observed that 8 out of 50 analyzed patients suffering from regional ileitis displayed anal fistulae as a possible complication. They were initially unaware that anal and perianal fistulae could present such a complication of ileitis^[8]. In 1952, Wells^[9] introduced the term *segmental colitis* while delivering a lecture at the Royal College of Surgeons of England. According to Wells, this condition is associated with the formation of fibrous tissue on the bowel wall leading to its increased thickening as well as the presence of ulcers of the mucosa. These ulcers have a patchy pattern of spreading and are therefore known as "skip lesions"^[9]. Most importantly this condition was observed in patients without lesions present in the ileum or jejunum. Wells presumed that segmental colitis is a form of colonic CD, but this was never acknowledged by Crohn himself^[9].

At present, research on CD has partitioned it into three categories: inflammatory, obstructive, and fistulating^[10]. The inflammatory and obstructive types tend to occur simultaneously and cause obstructions of the bowel due to thickening of the intestinal wall as a result of inflammation. The fistula types are commonly associated with erosion of the bowel walls including perianal fistulas and enteroenteric fistulas^[10]. Depending on the locations of these erosions the disease is called Crohn's or granulomatous colitis if symptoms occur in the large intestine, Crohn's enteritis if symptoms occur in the small intestine, or Crohn's ileitis if symptoms occur in the ileum^[11]. Furthermore, it has been documented that some patients suffer with inflammation of fat cells under the skin (erythema nodosum), or in large joints (peripheral arthritis)^[10]. It is important to note that CD is quite similar to another IBD known as ulcerative colitis (UC). The latter affects only the colon whereas CD can affect any region of the GI tract^[12].

WHO IS AFFECTED BY IBD?

Statistical evidence has indicated that the highest prevalence of CD and UC is in North America, northern Europe, and the United Kingdom. These diseases are beginning to rise in southern Europe, Asia, Africa, and Latin America. In fact, as much as 1.4 million persons in the United States and approximately 2.2 million individuals worldwide cope with IBD on a daily basis^[13]. However, in one epidemiological study of CD based on ethnicity, it was revealed that CD is least prevalent in African Americans, Asians, and Hispanics. The rate of prevalence for African Americans was 29.8 per 100000, for Hispanics it was 4.1 per 100000, and for Asians it was 5.6 per 100000^[14]. It has also been found that the occurrence of IBD is higher in industrialized countries such as North America and Europe *vs* under developed or developing countries. Therefore, this suggests that the pathogenesis of IBD may be caused by certain environmental factors^[15]. This indicates that genetic susceptibility alone cannot account for the prevalence of CD. In addition, the incidence and prevalence of CD is essentially equal among men and women. Unfortunately, CD is a lifelong debilitating disease which can start in early childhood and continue into late adulthood. Most cases of CD are usually reported or initially diagnosed when the patient is in his or her late teens or early twenties. Recent studies have indicated that in the last few decades the number of CD patients diagnosed before the age of 40 years has increased to 80%^[16]. This therefore emphasizes the young adult and adolescent age group as a primary target of this disease. Understanding the etiology of CD may facilitate the development of rapid and cost effective methods for disease diagnosis.

DIAGNOSIS OF CD

The most accurate and effective examination for the diagnosis of CD is a full colonoscopy along with intubation of the ileum^[17]. This type of endoscopic examination allows the physician to clearly visualize the colon, ileum, and even certain parts of the lower regions of the small intestine. Physicians can also take multiple biopsies of all the segments of the colon as well as the terminal ileum^[17]. Dye-based chromoendoscopy is an advanced imaging technique which allows for the visualization of subtle changes in the lining of the intestine. An alternative imaging method that can be utilized is capsule endoscopy, which is usually selected when there is no evidence of stricture or stenosis^[18]. Other technology detects inflammation of the distal ileum such as enhanced gadolinium magnetic resonance imaging which has been proven to be very effective in distinguishing between inflammatory diseases of the GI tract, is non-invasive, and does not produce any radiation^[19].

WHAT IS THE ETIOLOGY OF CD?

Unfortunately, the etiology or cause(s) of CD are still

unknown. However, there have been several theories that have been proposed to explain this phenomenon. For example, the leading theories suggest that CD can be caused by certain environmental factors or by a dysregulated immune response in a genetically susceptible host. Many believe a milieu of environmental factors such as diet and certain infectious agents may trigger this disease. For example, it has been found that a diet of refined sugars, fatty acids, fast foods, and minimal consumption of fruits, vegetables, and fibers can contribute to triggering the disease^[20]. Certain foods play a pivotal role in influencing the microbiome composition of the human gut. In fact, a "Westernized" diet is believed to change the microbiological environment such that there is an increased susceptibility for the development of intestinal bowel disease^[20]. Some of the infectious causative agents studied in connection with CD include viruses, yeast, and bacteria including *Escherichia coli*, *Listeria monocytogenes*, *Chlamydia trachomatis*, *Pseudomonas maltophilia*, *Bacteroides fragilis*, *Mycobacterium kansasii*, and MAP. Fortunately, it is almost universally accepted that a host genetic predisposition is critical for development of CD^[21].

In recent years, the amount of interest and research data in support of a possible infectious etiology for CD has been well noted. More specifically, the forerunner of the proposed infectious causative agents is MAP. However, there are several critics and skeptics who still discredit this theory. Therefore the goal of this review article is to shed light on this current predicament with the intention to further clarify our understanding of the pathogenesis of CD from the perspective of an infectious agent such as MAP.

MAP AND JOHNE'S DISEASE

It was in 1895 when Johne and Frothingham first identified MAP as the causative agent of chronic inflammation in the gut of a cow^[22]. Johne's disease was later coined after Johne for his work in identifying this chronic inflammatory enteric disease in cattle, but this disease has also been observed over the years in several different animals such as sheep, goats, rabbits, monkeys, and even chimpanzees^[22]. MAP belongs to the *Mycobacterium avium* complex (MAC) which consists of at least *M. avium* and *M. intracellulare*^[23]. Through DNA sequence analysis it is possible to evaluate the similarities and differences among mycobacterial strains. It has been documented that MAP shares certain sequence similarities with other strains of MAC. For example there is a 16S-23S rDNA internal transcribed spacer (ITS) that is approximately a 280 base paired region located on the rRNA operon of mycobacteria^[24]. It was found that ITS sequence analysis of MAP taken from 3 different mammalian species-bovine, primate, and human did not indicate much sequence variation between them and in 17 strains of MAC^[24]. Thus, the connection between MAP and other mycobacterial strains is observed through this highly conserved sequence similarity. In addition, mycobacteria can be broadly classified as either an environmental or parasitic

species on the basis of their epidemiological and pathological nature^[25]. Environmental mycobacteria such as the other strains of MAC can be considered as opportunistic bacteria that are found in a variety of habitats. Some of these environments include wet soil, rivers, agricultural slurry, the intestines of birds, ruminants, humans, and even within protists^[26,27].

MAP is an obligate parasitic mycobacterium that causes chronic inflammation in the gut of several mammalian species and is considered to have three major genetic differences that serve to separate it from other non-pathogenic MAC. These differences are the presence of an insertion element designated as IS900, the presence of a genetic element known as "GS", and the presence of a unique MAP gene (*bspX*) located in a specific genomic region. MAP contains a highly conserved insertion sequence (IS) or IS element referred to as IS900, which is repeatedly found in its genome approximately 15-20 times^[28]. IS900 contains 1451 base pairs and harbors neither terminal inverted repeats nor flanking direct repeats normally found in other classical IS elements^[28]. As a result, IS900 is grouped in a family of insertion elements that is specifically found in certain microorganisms. Some of these IS elements include IS901 and IS902 found in *Mycobacterium avium* subsp. *silvaticum*^[29], IS116 present in *Streptomyces clavuligerus*^[30], and IS1110 located in *M. avium*^[31]. It has been documented that the pathogenic nature of several microorganisms has been linked to the presence of IS elements^[32]. IS900 is able to take control of the translational machinery of MAP and thereby affects the expression of certain genes. It achieves this task by encoding for a putative transposase of 399 amino acids in size called p43 on one strand^[33]. On the complementary strand IS900 encodes for a very unique gene called the *hed* (host-expression-dependent) gene^[34]. This gene is quite unique in that upon entry into the MAP genome it requires a promoter, termination codon, and ribosome binding site (RBS). Previous studies have indicated that IS900 enters the genome of MAP at a specific consensus target sequence such that it is located between the RBS and start codon of the target gene in one specific direction^[33]. As a result of this alignment, the *hed* ORF comes under the control of the mycobacterium host promoter thereby allowing for the translation of the Hed protein^[33]. Thus, this is one probable explanation for how the insertion element IS900 may assist in the pathogenic phenotype of MAP compared with the other strains of MAC.

The second major genetic difference between MAP and other mycobacterial strains of MAC is that MAP contains a genetic element designated as "GS", which contains a low guanosine and cytosine (G + C) content^[35]. GS is a 6496 bp element which possesses six genes-*gsa*, *gsbA*, *gsbB*, *gsc*, *gsd*, and *mpa*^[36]. In addition, it has been found that the *mpa* gene of the GS element in MAP is a putative acetyltransferase, and has *mpa* homologues present in other microorganisms such as *oafA* and *oac* of *Salmonella typhimurium* and *Shigella flexneri*, respectively^[37-39]. Other virulence regions including "pathogenicity islands" or Pais

have been reported on MAP chromosome^[37] and have been found to be similar to a few protein-coding genes found in *Mycobacterium tuberculosis*. These protein-coding genes are *drdA*, *drdB*, and *drdC*, are located at Rv2936-Rv2938, and have been commonly associated with the pathogenic phenotype observed in *M. tuberculosis*^[38].

TRANSMISSION OF MAP TO HUMANS THROUGH COW'S MILK

The real concern for the transmission of MAP from cattle to humans is that MAP-infected cows remain asymptomatic in a lengthy subclinical phase^[39]. As a result of this, infected cows are not removed and may continue to be harvested for milk and meat, and the spread of MAP can go unnoticed through fecal matters to the rest of the herd^[40]. There have been several cases reporting the culture and isolation of MAP from the milk of subclinical or asymptomatic cows^[39].

There has been a plethora of documentation about the number of cases in several countries reporting outbreaks of human illness due to improper 'heat-treated' milk and dairy products. It has been observed that certain pathogens such as *Campylobacter* species, *Salmonella* species, *L. monocytogenes*, and even *Y. enterocolitica* have been found in pasteurized milk, powdered milk, and even cheese, thereby contaminating these products and causing human illness^[41]. Thus, it is apparent that milk can serve as a means of transmission of these pathogens. Similarly because MAP is found to a large extent in dairy herds and domestic livestock, it can be inferred that it may be present in raw milk. It is assumed that the pasteurization process will destroy any viable pathogens including MAP. However, there have been numerous case studies indicating the thermal-resistant characteristics of MAP thereby enabling its survival after pasteurization. Chiodini and Hermon-Taylor simulated pasteurization methodologies under laboratory conditions as defined by the Public Health Service, US Food and Drug Administration^[42]. They performed the high-temperature, short-time (HTST) method of pasteurization in which the milk samples were heated to 72 °C for 15 s in accordance with commercial pasteurization techniques^[43]. The results indicated that approximately 3%-5% of strains of MAP survived this process. Also, the pasteurization of MAP obtained from human tissues suspended in milk showed to have a higher survival rate (38.7% and 26.2%) than the bovine samples (8.7% and 9.0%)^[43]. Grant *et al*^[44] reported that MAP was not completely destroyed after pasteurization if it was already present in the milk at a concentration greater than 10⁴ cfu/mL. In other studies, Sung *et al*^[45] were able to statistically determine the *D* values for various strains of MAP tested which estimated that MAP can survive HTST pasteurization methods when initially present at a concentration greater than 10¹ organisms/mL of milk. However, there have been some critics who have dismissed the validity of the previous studies because they claimed that the HTST pasteuriza-

tion method performed in the laboratory setting cannot simulate commercial pasteurization conditions such as the turbulent flow of milk^[44]. For example, Stabel *et al*^[46] challenged the validity of these previous studies and reported that there was no evidence indicating the presence of viable MAP after the performance of HTST pasteurization simulated with an Armfield HTST laboratory pasteurizer. However, Grant *et al*^[44] defended the studies previously performed in the field and criticized the methodology selected by Stabel *et al*^[46]. She indicated that Stabel *et al*^[46] had frozen and sonicated MAP prior to its addition to raw milk. Grant *et al*^[44] also expressed that MAP will not under normal circumstances naturally undergo such treatments prior to contaminating milk samples. Furthermore, freezing and sonicating MAP will only make it more susceptible to heat shock. As already indicated by Richards *et al*^[47] in 1977, freezing (-70 °C) of bovine fecal samples contaminated with MAP substantially reduced the viability of MAP. Also, it was Sung *et al*^[45] who reported the decreased thermal resistance of declumped MAP cells compared to clumped MAP cells, thereby highlighting the changes caused by the sonication of MAP cells. It is without a doubt that milk can serve as a vehicle for the transmission of MAP from animals to humans through consumption of dairy and meat products from infected animals.

PREVALENCE OF MAP IN THE ENVIRONMENT AND IN WATER

One of the major contributors to the spread of MAP in the environment is through the feces of infected cattle. Both subclinically and clinically infected cows excrete massive amounts of MAP through their feces on various pastures and farmlands^[48,49]. This is a serious problem because it has already been documented that MAP can persist in the environment for long durations^[50]. MAP is capable of surviving in fecal matter and in the soil for up to 12 wk^[51]. Muskens *et al*^[52] conducted a study investigating whether infected cattle could transmit MAP to other animals such as sheep grazing on the same pastures. They reported that 20% (10/50) of sheep showed evidence for the presence of MAP in their tissues. Subsequently, MAP can spread and infect other animals which come in contact with infected cattle. Furthermore, the prevalence of MAP in the environment is not only due to infected cattle, but can be due to other infected animals such as rabbits and deer which can also spread MAP abundantly through their feces^[53]. Unfortunately, this is only part of the problem. In most cases the cow's fecal matter is used to make manure which is subsequently distributed across agricultural lands as fertilizer and thus contaminating ground water, rivers, and other surface bodies of water^[56]. It will be just a matter of time before the accidental host (human population) is infected with MAP. MAP has been shown to resist chlorine disinfection treatment at concentrations similar to those used to disinfect public drinking water systems^[54]. Clearly, it is apparent that water is a very

potent vehicle for the transmission of MAP to humans.

MAP CHALLENGES IN THE LABORATORY

From the outset, MAP is an obligate intracellular bacterium which presents multiple challenges in the laboratory with respect to its cultivation from tissue samples from both CD patients and even Johne's disease in animals. Unfortunately this fastidious bacterium is very slow-growing and often requires the cultures to be incubated for an extended period as much as 16 wk at a time^[55]. As a result it has become quite problematic over the years to isolate and culture it through conventional means. Furthermore, MAP has very specific growth requirements which must be met for its survival. For example, this intracellular bacillus is unable to synthesize iron-chelating compounds, and therefore its host must provide iron for MAP to survive. Furthermore, due to its high mycolic content mycobacteria can easily adapt to intracellular growth in macrophages and may even become drug resistant^[56]. In addition, MAP in CD assumes a cell-wall deficient spheroplast-like form which complicate culture requirement and void the use of the golden standard Ziehl-Neelsen mycobacterial staining test. For this reason, MAP in its spheroplastic form cannot be identified by light microscopy which adds to the challenges of confirming its presence in a laboratory setting^[57]. Due to these difficulties, MAP-specialized scientists looked towards better techniques for the detection and characterization of microorganisms. This led them to the utilization of IS900 polymerase chain reaction (PCR) for the detection of MAP and later on the development of appropriate culture media. Nevertheless, some challenges remain including standardization of the methodology, and most importantly spreading the awareness to clinicians and scientists that standard methodology is not appropriate for the detection of MAP in humans^[58].

INVESTIGATING MAP ASSOCIATION WITH CD

It was in 1913, when Dalziel (1861-1924), a surgeon at Glasgow reportedly characterized 13 cases of chronic intestinal enteritis in humans^[2]. Upon histological and clinical examination of nine patients, Dalziel specifically noticed that different parts of the gastrointestinal tract were affected: the jejunum, transverse and sigmoid colon, as well as the mid-ileum^[2]. He reported that these symptoms closely paralleled clinical findings observed in cattle suffering from Johne's disease, a chronic inflammatory disease of the gut. As a result, Dalziel speculated that *paratuberculosis*, the then known causative agent of Johne's disease, could be a potential etiological agent responsible for the observed symptoms in his patients^[2]. It was not until 1932 when Crohn's disease was first introduced as a clinical entity was it possible to connect the pathological and clinical findings described in CD to Dalziel's observations in 1913. However, much skepticism and uncertainty

Table 1 Studies supporting *mycobacterium avium* subspecies *paratuberculosis* association with Crohn's disease by Culture *n* (%)

Study	Crohn's disease	Control
Bull <i>et al</i> ^[63]	14 (33)	3 (33)
Chiodini <i>et al</i> ^[82]	16 (26)	13 (26)
Chiodini <i>et al</i> ^[59]	3 (100)	NP
Collins <i>et al</i> ^[64]	15 (19)	3 (6.3)
Gitnick <i>et al</i> ^[65]	4 (14.8)	1 (1.8)
Kirkwood <i>et al</i> ^[66]	4 (40)	0 (0)
Markesich <i>et al</i> ^[61]	12 (50)	1 (7.7)
Mendoza <i>et al</i> ^[67]	30 (100)	0 (0)
Moss <i>et al</i> ^[68]	6 (33.3)	1 (16.7)
Naser <i>et al</i> ^[69]	2 (100)	0 (0)
Naser <i>et al</i> ^[58]	14 (50)	0 (0)
Schwartz <i>et al</i> ^[62]	10 (37)	2 (5.6)
Sechi <i>et al</i> ^[57]	19 (63.3)	3 (10.3)
Singh <i>et al</i> ^[70]	4 (80)	6 (27.3)
Singh <i>et al</i> ^[71]	29 (50)	5 (12.5)
Wall <i>et al</i> ^[72]	6 (20)	0 (0)

NP: Not performed.

persisted with respect to the etiology of Crohn's disease. Furthermore, confidence in this mycobacterial hypothesis over the years has suffered tremendously due to the substantial difficulty and failure in culturing mycobacteria from CD tissues and the reliance on methodology which were not appropriate for MAP from humans. MAP association with CD theory was revived when Chiodini *et al*^[59] in 1984 reported the isolation of uncharacterized mycobacteria from tissues of three CD patients. They proposed that the bacterium existed in a cell-wall defective form which was later characterized as MAP^[60]. Similar results were reported from studies out of David Graham and John Hermon-Taylor laboratories (discussed below). Advancements in cultural techniques and PCR assays unique to MAP by Naser's team (discussed below) fueled and renewed interest in investigating a possible etiological connection between MAP and CD.

CULTURE OF MAP FROM CD PATIENTS

In this review, data from a total of 23 peer review studies which investigated the presence of MAP in CD specimens using culture techniques were reviewed. As shown in Table 1, the results from 16 (70%) studies supported the association between MAP and CD. Only 7 (30%) studies did not support such association (Table 2). Much of the difficulty in culturing or isolating MAP stems from the fact that this fastidious organism has very specific nutritional requirements and is a very slow growing bacterium^[59,61,62]. Culture of MAP in liquid or agar-based media requires weeks to months of laboratory incubation^[22]. The presence of MAP in a cell wall-deficient spheroplastic form in humans adds additional challenges to growing it in the laboratory. Many investigators reported the recovery of MAP in a cell wall-deficient form from the tissues of CD patients at a higher occurrence than control groups consisting of non-IBD patients^[59,61-72]. Certainly,

Table 2 Studies not supporting *mycobacterium avium* subspecies *paratuberculosis* association with Crohn's disease by Culture *n* (%)

Ref.	Crohn's disease	Control
Clarkston <i>et al</i> ^[83]	0/21 (0)	NP
Dumonceau <i>et al</i> ^[105]	0/31 (0)	0/22 (0)
Graham <i>et al</i> ^[84]	6/19 (31.5)	7/17 (41)
Kallinowski <i>et al</i> ^[75]	0/21 (0)	0/24 (0)
Kreuzpaintner <i>et al</i> ^[85]	0/23 (0)	0/23 (0)
Parrish <i>et al</i> ^[73]	0/130 (0)	0/130 (0)
Ricanek <i>et al</i> ^[74]	2/75 (2.7)	2/135 (1.5)

NP: Not performed.

the advent of PCR, RT-PCR and nested PCR had facilitated the detection of MAP IS900 in cultures from CD patients^[53,57,58,64,66,68,69,72]. Table 1 lists a total of 16 studies which strongly support the association between MAP and CD. The development of mycobacterial growth indicator tube (MGIT) sparked a new wave of interest led by Saleh Naser team who supplemented MGIT media with additives essential for survival of cell wall-deficient *in vitro* and restoration of the cell wall. Consequently, Schwartz *et al*^[62] reported a higher frequency of MAP in CD patients at 37% (10/27) *vs* healthy controls at 5.6% (2/36). What is truly insightful in this study is the fact that MAP was found at a higher percentage (86%) in surgically resected tissue samples than in tissue biopsies (20%) taken from CD patients^[62]. These results alluded to the supposition that MAP may in fact be located below the mucosal layer instead of found on the apical surface area^[62]. Naser *et al*^[69] further employed the same culture condition to study whether or not MAP is present in human milk. They reported the presence of MAP in 100% (2/2) of breast milk samples taken from lactating CD mothers who had just given birth, compared to 0% (0/5) of healthy lactating controls. Thus, this study provides critical evidence to support the similarity between Johne's disease and MAP infection in CD. MAP was later on detected from breast milk from additional CD patients (data not shown). Most interestingly, Naser *et al*^[58] were able to culture viable MAP from the buffy coat of blood sampled from CD patients at a significant percentage 50% (14/28). These intriguing results are further substantiated based on the fact that there was no evidence for the culture of MAP from the blood of the healthy control groups 0% (0/15). Other scientists reported the presence of MAP in 14/33 (42%) bowel-pinch biopsies of CD patients (14/33) compared to 3/33 (9%) non-IBD controls. It was Kirkwood *et al*^[66] who sought to investigate if there was an association between MAP and CD in children who were symptomatic of this disease at an early stage. They revealed that 40% (4/10) of the cultured mucosal biopsies from the CD patients contained viable MAP, whereas 0% (0/4) of the healthy non-IBD controls showed no evidence for the presence of MAP. Consequently, these findings clearly indicate the possible association between MAP and CD, and according to Kirkwood *et al*^[66] these results

Table 3 Studies supporting *mycobacterium avium* subspecies *paratuberculosis* association with Crohn's disease by polymerase chain reaction *n* (%)

Ref.	Crohn's disease	Control
Autschbach <i>et al</i> ^[76]	52 (100)	5 (100)
Bentley <i>et al</i> ^[86]	122 (33.8)	43 (21.5)
Bull <i>et al</i> ^[63]	34 (92)	9 (26)
	14 (42)	3 (9)
Collins <i>et al</i> ^[64]	15 (19)	3 (6.3)
Dell'Isola <i>et al</i> ^[87]	13 (72)	7 (29.2)
Erasmus <i>et al</i> ^[88]	10 (38)	4 (11)
Fidler <i>et al</i> ^[89]	4 (12.9)	0 (0)
Gan <i>et al</i> ^[90]	17 (47.2)	3 (15)
Ikonomopoulos <i>et al</i> ^[91]	7 (35)	NP
Kirkwood <i>et al</i> ^[66]	22 (39)	6 (15)
	8 (16)	0 (0)
Lisby <i>et al</i> ^[92]	11 (46)	3 (11)
Mendoza <i>et al</i> ^[67]	18 (60)	0 (0)
Mishina <i>et al</i> ^[78]	8 (100)	0 (0)
Moss <i>et al</i> ^[68]	6 (33.3)	1 (16.7)
Murray <i>et al</i> ^[93]	2 (22)	0 (0)
Naser <i>et al</i> ^[69]	2 (100)	0 (0)
Naser <i>et al</i> ^[58]	13 (46)	3 (20)
Romero <i>et al</i> ^[77]	10 (83)	1 (17)
Ryan <i>et al</i> ^[94]	6 (50)	0 (0)
Sanderson <i>et al</i> ^[95]	26 (65)	5 (12.5)
Scanu <i>et al</i> ^[96]	20 (87)	3 (15)
Sechi <i>et al</i> ^[57]	25 (83.3)	3 (10.3)
Singh <i>et al</i> ^[70]	4 (80)	5 (22.7)
Singh <i>et al</i> ^[71]	28 (96.6)	NP
Tiveljung <i>et al</i> ^[97]	3 (27)	0 (0)
Tuci <i>et al</i> ^[98]	21 (68)	11 (48)
Wall <i>et al</i> ^[72]	6 (20)	0 (0)

NP: Not performed.

imply that MAP maybe implicated with the early-onset of CD in children. Sechi *et al*^[57] also reported a particularly strong association between MAP and CD based on their population study which involved the analysis of people in Sardinia diagnosed with and without CD. According to their results it was found that MAP DNA was detected in intestinal mucosal biopsies of approximately 63% (19/30) of CD patients compared to 10.3% (3/29) of control patients.

Contrary to the above data, there have been some studies providing evidence for the dismissal of MAP as a causative agent of CD (Table 2). For example, Parrish *et al*^[73] conducted a study analyzing blood samples taken from 260 individuals who consisted of 130 CD patients and 130 healthy individuals. After culturing MAP, the results revealed that none of the CD patients 0% (0/130) as well as the healthy controls 0% (0/130) showed evidence for the presence of MAP^[73]. Only one patient was reported having a positive result by PCR^[73]. Due to the fact that MAP and MAP DNA are present in the food chain and the fact that MAP DNA has been detected in the blood of patients with CD and type I diabetes mellitus and in less frequency in the blood of healthy controls, most scientists in the field may question the protocol used in this study. In another study, Ricanek *et al*^[74] collected bowel biopsies from 321 individuals,

of which 75 of these biopsies were collected from CD patients and 135 were collected from non-IBD patients. After long-term culture of MAP it was reported that only 2.7% (2/75) of CD patients and 1.5% (2/135) of non-IBD patients showed the presence of MAP^[74]. Similarly, Kallinowski *et al*^[75] documented the inability to culture MAP from a variety of sources such as stool, sera, and even gut tissue samples. They reported that 0% (0/21) of CD patients and 0% (0/24) of healthy controls had MAP through culture^[75]. The results from these studies should not be surprising since MAP is extremely fastidious and requires specialized culture media to grow which is contrary to culture media used in these studies. Other studies which failed to detect MAP in CD have depended on traditional standard methodology designed to culture and detect bacillary MAP from Johne's disease animals or other *Mycobacterium* species. It is important that investigators realize that *M. avium* subspecies *paratuberculosis* is not the same as *M. avium* or *M. tuberculosis*. Moreover, tissue and blood specimens collected from patients with active antibiotic treatment should be used for attempts to culture MAP in the laboratory. Rarely did the studies described in Table 2 allotted to whether the subjects used in their studies had antimicrobial agents prior to submission of the specimens.

DETECTION OF MAP DNA BY PCR

A total of 52 studies investigating MAP DNA in CD have been reviewed. Table 3 lists a total of 27 studies providing evidence in support of MAP association with CD by PCR. On the contrary, Table 4 lists 25 studies which present data in contradiction of MAP-CD association.

One of the studies showing a strong connection between MAP and CD has been performed by Autschbach *et al*^[76]. They reported that a staggering 52% (52/100) of tissue from CD patients were found positive for the presence of MAP DNA compared to only 5% (5/100) of the non-IBD patients. Similarly, Romero *et al*^[77] had examined several surgical tissue samples from 20 individuals by performing nested PCR specific for the IS900 sequence. The results from Naser's lab indicated that a substantially high percentage 83% (10/12) of CD patients were positive for the presence of MAP, while a much smaller percentage 17% (1/6) of non-IBD patients were positive for MAP^[77]. In addition, there was another compelling study conducted by Bull *et al*^[63] in 2003 in John Hermon-Taylor's laboratory, which presented data in support of MAP as a causative agent for CD. Fresh ileocolonic mucosal biopsies were collected and analyzed for the presence of MAP by the performance of PCR specific for IS900. The results revealed that 92% (34/37) of CD patients were positive for the presence of MAP DNA compared to a significantly diminished number of healthy controls 26% (9/34)^[63]. In this same study Bull *et al*^[63] had cultivated MAP using MGIT cultures described by Naser *et al*^[58] and Schwartz *et al*^[62]. After twelve weeks of incubation, PCR was performed with these cultures which again indicated

Table 4 Studies not supporting *Mycobacterium avium* subspecies *paratuberculosis* association with Crohn's disease by polymerase chain reaction *n* (%)

Ref.	Crohn's disease	Control
Al-Shamali <i>et al</i> ^[99]	0 (0)	0 (0)
	0 (0)	0 (0)
Baksh <i>et al</i> ^[100]	0 (0)	NP
Bernstein <i>et al</i> ^[101]	0 (0)	6 (21.4)
Cellier <i>et al</i> ^[102]	2 (4)	2 (10)
	0 (0)	0 (0)
Chiba <i>et al</i> ^[103]	0 (0)	0 (0)
Clarkston <i>et al</i> ^[83]	1 (4.8)	0 (0)
Dumonceau <i>et al</i> ^[104]	17 (47)	13 (57)
	0 (0)	0 (0)
Domonceau <i>et al</i> ^[105]	0 (0)	0 (0)
Ellingson <i>et al</i> ^[106]	0 (0)	0 (0)
Frank and Cook ^[81]	0 (0)	0 (0)
Gibson <i>et al</i> ^[107]	0 (0)	0 (0)
Kallinowski <i>et al</i> ^[75]	0 (0)	0 (0)
Kanazawa <i>et al</i> ^[108]	0 (0)	0 (0)
Kreuzpaintner <i>et al</i> ^[85]	0 (0)	0 (0)
Lozano-Leon <i>et al</i> ^[109]	0 (0)	0 (0)
Parrish <i>et al</i> ^[73]	0 (0)	1 (0.77)
Ricanek <i>et al</i> ^[74]	0 (0)	1 (0.28)
Riggio <i>et al</i> ^[110]	0 (0)	0 (0)
Quirke ^[21]	0 (0)	0 (0)
Rowbotham <i>et al</i> ^[80]	0 (0)	1 (3.8)
Sasikala <i>et al</i> ^[79]	0 (0)	0 (0)
Suenaga <i>et al</i> ^[111]	10 (100)	14 (87.5)
	10 (100)	14 (87.5)
Toracchio <i>et al</i> ^[112]	1 (5)	NP
Tzen <i>et al</i> ^[113]	0 (0)	3 (27.3)
Wu <i>et al</i> ^[114]	0 (0)	NP

NP: Not performed.

a higher frequency of CD patients 42% (14/33) positive for MAP DNA *vs* only 9% (3/33) of healthy controls^[63]. This data strengthens the support of MAP in connection with CD. Mishina *et al*^[78] analyzed mucosal specimens using RT-PCR for the detection of MAP RNA where they found MAP in 100% (8/8) of CD patients and 0% (0/2) in non-IBD. This study is of particular importance because MAP RNA was amplified (without culture) adding more support to the presence of viable MAP in CD^[78].

At the same time, many studies based on PCR techniques have failed to detect MAP DNA in CD and concluded the lack of association between MAP and CD (Table 4). For example, Sasikala *et al*^[79] indicated that 0% (0/93) of CD patients showed the presence of MAP and 0% (0/97) of healthy controls were also negative for the presence of MAP. Similarly, Rowbotham *et al*^[80] reported that none (0/68) of CD patients were positive for the presence of MAP and just 3.8% (1/26) of healthy controls had MAP. Lozano-Leon *et al*^[109] indicated the absence of MAP in the blood of 73 CD patients and 73 healthy controls. Frank and Cook in 1996 also reported the absence of MAP in both CD and control subjects^[81]. The investigators in these studies should be commended on their interest to question whether or not MAP is associated with CD, and for including impressive numbers of specimens in their studies. Due to the fact that MAP

and MAP DNA are found in the food chain including dairy and meat products as well as in drinking water, it is difficult to accept that MAP or MAP DNA is not detected even accidentally in some specimens. The methodology used in many of these studies must have lacked essential steps to recover the low abundance of MAP in CD specimens and must have not been able to reduce the laboratory loss of some MAP or MAP derivatives. Whether the loss of MAP occurred at the specimen collection level or during the analysis, it should be avoided. Tissue specimens must be collected appropriately and adequately from active ulcerated sites. Specimens should be transported promptly and appropriately by avoiding freezing and use of anti-microbial solutions. Blood should be withdrawn into tubes with anticoagulants, transported without freezing, and promptly, to avoid lysis of leukocytes and loss of MAP. DNA extraction conditions should be optimized to recover single MAP genome which is also free from PCR inhibitors such as hemoglobin. Earlier study in our laboratory suggested that MAP from CD patients contained limited IS900 copies compared to bovine MAP strains. Nested PCR consisting of two amplification rounds is necessary for sufficient detection of MAP DNA. For reasons mentioned above and other unknown factors, standard PCR based on a single amplification should not be used for detection of MAP in CD.

CONCLUSION

In this review, data has been presented in the form of tables providing evidence for and against an association between MAP and CD by PCR and culture. It was revealed that MAP can be detected and isolated from the tissues, blood, and milk of many CD patients. Based on this information, MAP is definitively involved in the pathogenesis of some CD cases even though other studies have not acknowledged this association as represented in Tables 2 and 4. It must be emphasized that much of the controversy concerning MAP and CD stems from the inconsistent methodologies that have been used in the detection and isolation of MAP, which have questioned the causal relationship between this bacterium and CD. These observed discrepancies result from the fact that the methods that were designed for the detection of MAP in animals with Johne's disease are inappropriate for the detection of MAP in humans. Consequently, the need for more sophisticated and optimized methodologies are required so that there can be accurate detection and isolation of MAP in CD patients. One such methodology has been developed in our laboratory, and success has been achieved based on key principles shown in Figure 1. Other factors that may also limit the detection of MAP in clinical samples from some CD patients include the stage of the disease, and prior treatment with antibiotics or drugs with antimicrobial activity. For example, negative detection of MAP in peripheral blood samples could be correlated with a localized intestinal CD com-

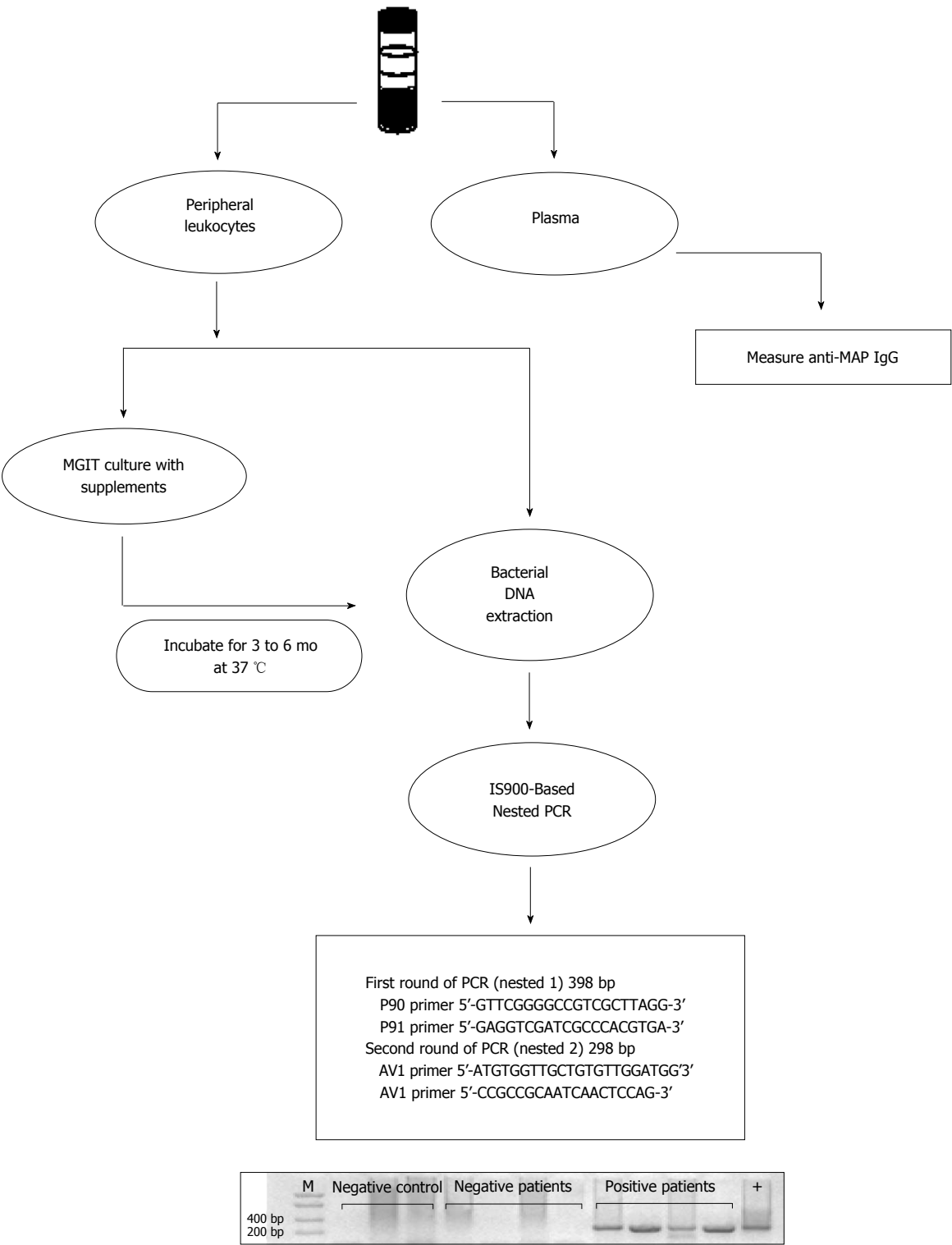


Figure 1 Schematic illustration of successful *Mycobacterium avium* subspecies *paratuberculosis* detection in clinical samples. Coded EDTA blood samples were collected from patients for investigating the presence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Blood plasma was analyzed by measuring the concentration of anti-MAP IgG antibodies. Peripheral leukocytes were analyzed for the presence of MAP. In the first method, DNA was extracted followed by IS900-based nested polymerase chain reaction (PCR) using MAP-specific primers. In the second method a mycobacterium growth indicator tube (MGIT) liquid culture system with supplements was used to culture MAP lacking cell wall followed by 3 to 6 mo incubation and IS900-based nested PCR analysis.

pared to cases with advanced disease associated with systemic complications. The latter is most likely to lead to the presence of MAP in circulation.

Finally, it is also worth noting that it is a fact that CD

is a syndrome with multifactorial etiology. It is very possible that lack of detection of MAP in clinical samples from some CD patients may be due to the absence of MAP role in these patients. The latter statement is con-

ditional on utilization of methodology appropriate for detection of human MAP strains. Stratification of CD and IBD patients for the presence or absence of MAP is necessary for appropriate and effective treatment which may lead to a cure.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease***Escherichia coli*-host macrophage interactions in the pathogenesis of inflammatory bowel disease**

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Abstract

Multiple studies have demonstrated alterations in the intestinal microbial community (termed the microbiome) in Crohn's disease (CD) and several lines of evidence suggest these changes may have a significant role in disease pathogenesis. In active and quiescent disease, both the faecal and mucosa-associated microbiome are discordant with matched controls with reduced biodiversity, changes in dominant organisms and increased temporal variation described. Mucosa-associated adherent, invasive *Escherichia coli* (*E. coli*) (AIEC), pro-inflammatory and resistant to killing by mucosal macrophages, appear to be particularly impor-

tant. AIEC possess several virulence factors which may confer pathogenic potential in CD. Type-1 pili (FimH) allow adherence to intestinal cells *via* cell-surface carcinoembryonic antigen-related cell adhesion molecules and possession of long polar fimbriae promotes translocation across the intestinal mucosa *via* microfold (M)-cells of the follicle-associated epithelium. Resistance to stress genes (*htrA*, *dsbA* and *hfq*) and tolerance of an acidic pH may contribute to survival within the phagolysosomal environment. Here we review the current understanding of the role of mucosa-associated *E. coli* in Crohn's pathogenesis, the role of the innate immune system, factors which may contribute to prolonged bacterial survival and therapeutic strategies to target intracellular *E. coli*.

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Key words: Crohn's disease; Inflammatory bowel disease; *Escherichia coli*; Intra-macrophage survival and replication; Phagolysosome; Autophagy

Core tip: There is significant evidence implicating adherent, invasive mucosa-associated *Escherichia coli* (AIEC) in the pathogenesis of Crohn's disease. AIEC translocate M-cells of Peyer's patches and lymphoid follicles of the colon, and then to survive and replicate within underlying mucosal macrophages. How Crohn's AIEC resist killing and adapt to the environment within the phagolysosome to survive and grow within macrophages is still poorly understood. Here we review the current understanding of the role of AIEC in Crohn's pathogenesis, the role of the innate immune system, factors which may contribute to prolonged bacterial survival and therapeutic strategies to target intracellular AIEC.

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INTRODUCTION

Crohn's disease (CD) is a chronic relapsing inflammatory bowel disease (IBD) of multifactorial aetiology, affecting any part of the gastrointestinal tract from mouth to anus. Patients typically suffer from abdominal pain, diarrhoea and weight loss which may be associated with extra-intestinal manifestations including erythema nodosum, iritis and arthritis. The intestinal pathological findings are characterised by transmural inflammation, deep mucosal ulcers, abscesses, fissures and granuloma formation^[1]. These chronic inflammatory lesions are proposed to develop due to a disrupted intestinal barrier, Paneth cell dysfunction and a disturbed innate immune response, resulting in the accumulation of antigen-presenting cells (such as dendritic cells and macrophages), lymphocytes and plasma cells within the intestinal mucosal layer^[1,2]. Pathological characteristics resemble the mucosal lesions and intestinal inflammation elicited by known enteric gut pathogens such as *Shigella* and *Salmonella* spp^[3].

CD is classically described to have a bimodal incidence with the highest rates seen in adolescents and young adults and a second peak in later years, although this has recently been questioned^[4]. It is associated with a small increase in mortality (standardised mortality ratio 1.52) but very considerable morbidity, disrupting work, study and family life^[5]. Historically approximately 80% of cases needed surgery at some time^[6] but the use of immunosuppressants and biologics has increased and is associated with a reduced 5 years risk of major surgery^[7]. The condition is more common in Europe and North America^[8]. However, incidence is rapidly increasing worldwide particularly in developed nations adopting a western style diet, as seen in Japan^[9]. Likewise, those emigrating from poor and developing nations to the West, within a few years of moving are at increased risk of developing CD presumably due to a key change in their lifestyle and environment^[10].

The gut microbiota plays an essential role in the shaping of the intestinal immune response in healthy individuals^[11]. There is now very strong evidence that both a reduction in the numbers of beneficial bacteria and increases in numbers of harmful bacteria living naturally in the gut are present in CD^[12] although it is less clear which of these changes might be causative and which might be a consequence of inflammation. Several independent groups have consistently shown changes in both the faecal and mucosa-associated microbiome in Crohn's patients and unaffected relatives^[13-15], an imbalance referred to as "dysbiosis" (Figure 1). Changes are typified by reduced biodiversity and alterations in the dominant organisms, specifically reduction in beneficial firmicutes and increase in numbers of proteobacteria [including

Escherichia coli (*E. coli*)]^[14,16,17].

There is also clear evidence to suggest that a number of lifestyle factors contribute to the dysbiosis of gut microbiota observed in CD (see Figure 1). This includes key environmental triggers such as smoking^[18], with cessation abrogating the observed dysbiosis^[19]. Also a key risk factor in CD is a intake of a "westernised" diet, high in fat and sugar, low in fruit and vegetable fibre^[20]. In a mouse model with a humanised microbiota, a switch to a high fat, high sugar diet altered the microbiome within 1 d^[21]. A similar diet has also been observed to increase numbers of Proteobacteria, such as *Bifidobacteria*^[22] and mucosally adherent, invasive *E. coli* (AIEC)^[23].

GENETIC SUSCEPTIBILITIES IN BACTERIAL RECOGNITION, AUTOPHAGY AND PHAGOCYTE-SPECIFIC GENES IN CD

The recent identification of genes associated with CD has been informative in improving our understanding of its pathogenesis, highlighting impairment of genetic components essential for innate immunity, intestinal barrier integrity and in microbial recognition and clearance^[24] (see Figure 1). Following on from earlier work^[25,26], Genome-wide association studies have now identified 163 IBD risk loci, 30 of which are CD specific and 110 shared between ulcerative colitis and Crohn's^[27]. Identified polymorphisms in the innate immune system of Crohn's patients include genes that are linked to processes such as pathogen recognition [nucleotide-binding oligomerization domain-containing-2 (*NOD2*)/Crohn's-associated gene identified was Caspase-recruitment domain 15 (*CARD15*) and interleukin 23 receptor (*IL23R*)] and autophagy [immunity-related GTPase M (*IRGM*) and autophagy-related 16-like 1 (*ATG16L1*)], all relevant to killing of bacteria within macrophages^[24-26].

The first *CARD15* encoding the NOD2 receptor^[28,29]. Mutations in this gene probably account for about 15% of Crohn's causation in the West although there are geographical variations with a lesser effect in northern European countries and no apparent impact on CD causation in Japan^[30]. The NOD2/*CARD15* protein is part of the innate immune system and is expressed in the cytoplasm of macrophages and Paneth cells^[31]. CD-associated mutations in NOD2/*CARD15* affect the leucine-rich domain recognising the bacterial cell wall peptidoglycan component, muramyl dipeptide (MDP), of both Gram-positive and Gram-negative bacteria. After recognition, NOD2 activates nuclear factor kappa B and induces the production and release of proinflammatory cytokines. Crohn's-associated NOD2/*CARD15* mutations are considered to be loss of function mutations with evidence for reduced production of anti-bacterial defensins by Paneth cells and for a reduced IL-8 response to MDP by macrophages^[32]. In association with NOD2/*CARD15* mutations, polymorphism in genes *SLC22A4* and *SLC22A5*,

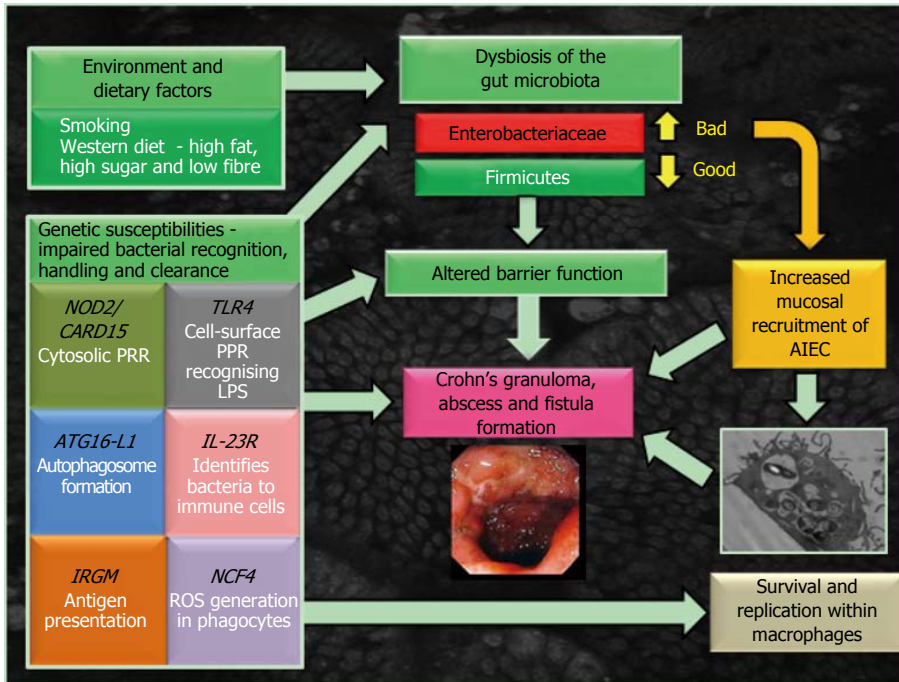


Figure 1 Model for the development of Crohn's disease. AIEC: Adherent, invasive *Escherichia coli*; ATG16L1: Autophagy-related 16-like 1; CARD15/NOD2: Caspase-recruitment domain 15/nucleotide-binding oligomerization domain-containing-2 receptor; IL-23R: Interleukin-23 receptor; IRGM: Immunity-related GTPase M; LPS: Lipopolysaccharide; NCF4: Neutrophil cytosolic factor-4 gene; PRR: Pathogen recognition receptor; ROS: Reactive oxygen species; TLR4: Toll-like receptor 4.

encoding the organic cation transporters OCTN1 and OCTN2 have also been identified with variants expressed in intestinal epithelial cells, T cells and macrophages^[33]. In addition, a mutation in two haplotypes of *DLG5*, encoding scaffolding protein, has also been confirmed to be associated with *NOD2/CARD15* mutations in Crohn's patients^[34].

Two other key genes associated with Crohn's are *ATG16L1* and *IRGM*^[35-37]. Both encode proteins that play a key role in autophagy, a cellular process facilitate not only disposal of protein aggregates, DNA, lipids and damaged organelles but also an integral step in the mechanism by which macrophages degrade, kill and clear invading phagocytosed bacteria (a process also termed xenophagy), including *Mycobacteria* and *Salmonellae*^[38-40].

Additional Crohn's susceptibility loci relevant to aberrant microbial recognition and handling and/or phagocyte function include toll-like receptor 4 (*TLR4*), leucine-rich repeat serine, threonine protein kinase-2 (*LRRK2*), neutrophil cytosolic factor-4 (*NCF4*) and *IL-23R*.

TLR4 is an apical cell-surface pathogen recognition receptor on intestinal epithelial cells, macrophages and dendritic cells, key in detection of lipopolysaccharide (LPS) presented on the outer-membrane surface of Gram-negative bacteria, with polymorphism of *TLR4* at D299G leading to hypo-responsiveness to LPS^[41]. *LRRK2* has been linked to CD through the association of a single-nucleotide polymorphism on chromosome 12q12^[26] and in murine studies where *LRRK2*-deficiency resulted in increased inflammation and significantly poorer clinical outcomes following administration of dextran sodium sulphate to induce colitis^[42]. The identification of *NCF4*

as a Crohn's susceptibility gene is also important^[36]. *NCF4* encodes the p40-phox subunit of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase crucial for reactive oxygen species (ROS) production by phagocytic cells in response to microbial infection, with molecular defects in NADPH oxidase already established to result in chronic granulomatous disease^[43]. Key studies show that altered neutrophil recruitment, along with an abnormal production of cytokines and reduced bacterial clearance, follow either acute trauma to the rectum and ileum^[44], or subcutaneous injection of heat-killed *E. coli* in Crohn's patients^[45]; (see Figure 2). Whilst these studies suggest macrophages may be involved in a key step of the observed immune dysfunction in CD, it is not yet clear whether this represents an inherent defect in macrophage function.

Variants of the *IL-23R* gene have also been linked to Crohn's^[46]. *IL-23R* is expressed by activated dendritic cells and macrophages, and *IL-23* can induce production of inflammatory cytokines that may contribute to intestinal inflammation^[47].

SPECIFIC BACTERIA IN THE PATHOGENESIS OF CD

There have been a number of distinctive studies that strongly favour the hypothesis that a specific bacterium plays a pivotal role in the initiation of chronic inflammation and development of CD. Early serological and culture studies suggested that *Mycobacterium avium* subspecies *paratuberculosis* (MAP), an obligate intracellular bacterium causing a chronic intestinal inflammatory disease

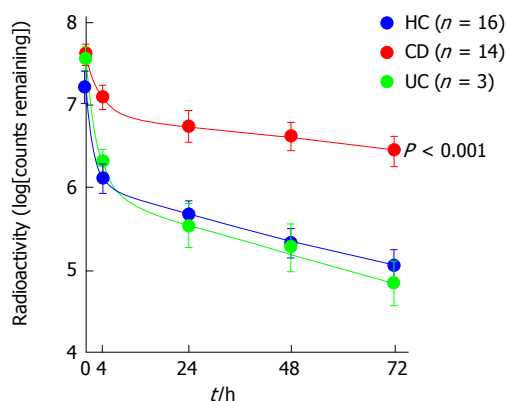


Figure 2 Patients with Crohn's disease exhibit reduced bacterial clearance of subcutaneously injected ^{32}P -labelled heat-killed *Escherichia coli* relative to healthy controls and patients with ulcerative colitis. Reproduced with permission. © 2009 Rockefeller University Press. Originally published in *Journal of Experimental Medicine* 206: 1883-1897^[45]. CD: Crohn's disease; HC: Healthy controls; UC: Ulcerative colitis.

in cattle (Johne's disease), was more prevalent in Crohn's patients^[48,49]. A study by Ryan and colleagues^[50] also confirmed the presence of MAP DNA in granulomatous lesions of CD patients. MAP-reactive CD4 T cells have also been found in patients with Crohn's^[51]. Even though, MAP has been hypothesised to be as contributing agent for Crohn's pathogenesis, there is still great controversy, and absence of conclusive evidence, to fully supporting this hypothesis^[52]. Our own studies have suggested perhaps that microbial mannan (present in yeast cell walls and Mycobacterium species such as MAP) may be a key environmental factor to suppress macrophage killing of intracellular bacteria^[53]. The shared susceptibility association of *NOD2* and *IL-23R* polymorphisms seen in both CD and Mycobacterial disease suggests MAP may yet be important in CD pathogenesis^[54].

Faecalibacterium prausnitzii may also be important with low levels strongly associated with early disease recurrence after intestinal surgery^[55]. This effect may be due to bacterial production of anti-inflammatory molecules with culture supernatant shown to reduce the severity of colitis in an animal model.

The finding of increased mucosa-associated *E. coli* in the sub-mucus niche or within the mucosa itself has proved particularly consistent in CD^[12]. Early serological studies described high antibody titres against *E. coli* in Crohn's patients compared to unaffected controls^[56] and this was later supported by immunohistochemical studies demonstrating *E. coli* antigens within macrophages in CD tissue^[57]. Many groups, including our own, have shown an increase in mucosa-associated *E. coli* in CD, both in the ileum and in the colorectum^[58-64]. We ourselves observed that aerobic culture of colonoscopic biopsies after removal of the mucus layer with dithiothreitol is often sterile in control colons whereas the colon in CD and colon cancer contains increased bacterial numbers in this sub-mucus niche, more than half of which are *E. coli*^[60], even though these organisms account for less than 1% of the faecal microbiota^[65]. Poor

correlation between site of inflammation and presence of *E. coli*^[63] and tendency to show that the same organisms can be identified from various sites within the same colon^[60,66] are compatible with the organisms having a causative role in the inflammation rather than merely colonising inflamed mucosa. Evidence for a primary pathogenic role is also given by their presence within granulomas^[67], the histological hallmark of CD, by their ability to induce granuloma formation *in vitro*^[68] and ability for similar *E. coli* to cause granulomatous colitis in dogs^[69], and potentially in cats and swine too^[70].

These *E. coli* pathovars associated with CD have been designated AIEC based on their ability to adhere to, and invade into, intestinal epithelial cell-lines, induce release of pro-inflammatory cytokines, and possess an ability to survive and replicate with intestinal macrophages^[71]. Phylogenetic analysis shows that most mucosa-associated *E. coli* isolated from the tissue of Crohn's patients belong to groups B2 and D^[65] as per extra-intestinal isolates, whereas most commensal *E. coli* strains would belong to group A^[72].

CROHN'S AIEC-HOST INTESTINAL MUCOSA INTERACTIONS

Aphthous ulcers of the "dome" or follicle-associated epithelium (FAE), overlying Peyer's patches in the distal ileum and lymphoid follicles of the colon, are likely the initial mucosal lesions occurring in Crohn's patients^[73-75], and have been observed in patients using magnifying chromoendoscopy^[76]. The FAE effectively forms the interface between the intestinal lymphoid system and the luminal environment. Specialized microfold (M) cells accounting for about 5% of cells in the FAE are optimized for antigen adherence and transport, and for immunological sampling of microorganisms^[77]. Several invasive bacteria take advantage of the transcytotic characteristics of M cells to use them to cross the gut, including *Yersinia*, *Salmonella* and *Shigella* spp^[78-80]. It was suspected that the portal of mucosal entry of AIEC was also likely through M cells^[81] and our recent studies successfully modelling M cells *in vitro*, demonstrated that Crohn's AIEC could indeed translocate through M cells (up to 20-fold compared with parent Caco2 cells) and through isolated human ileal FAE^[82]. Adhesion and subsequent translocation of AIEC across murine and human Peyer's patches, and across M cells *in vitro*, was observed to be dependent on possession of the *lpf* operon, encoding long polar fimbriae (Lpf) in AIEC^[83]. Isolates expressing *lpf* have been found to be more prevalent in Crohn's mucosae than that of non-IBD controls^[84]. *Ex vivo* studies also indicate a defective mucosal barrier to bacteria in the Peyer's patches from Crohn's patients^[85,86]. It is plausible therefore that increased bacterial load at M cells is important in the development of Crohn's. A striking correlation also exists between the age-related incidence of CD and the number of Peyer's patches in the small bowel, the latter peaking in late adolescence and then

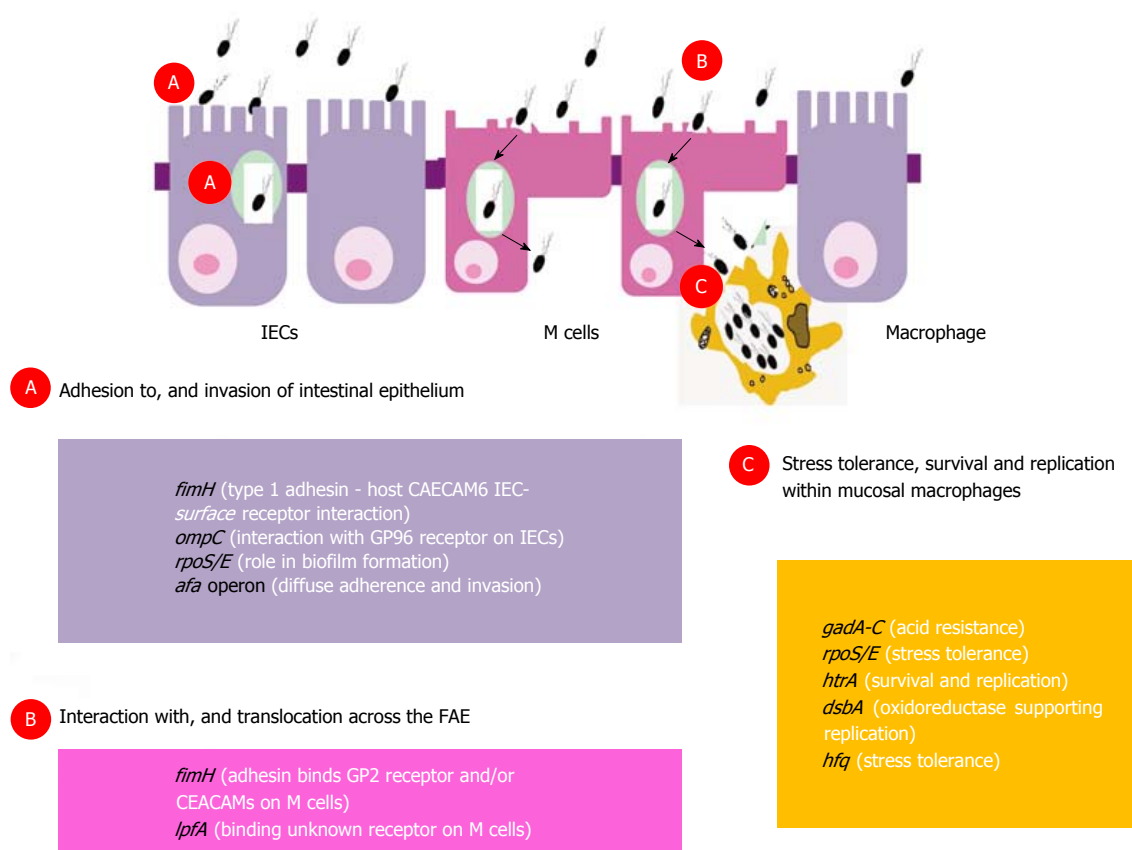


Figure 3 Crohn's mucosally associated adherent, invasive *Escherichia coli* host mucosa interactions: genotype-phenotype relationships. A: Adhesion to, and invasion of intestinal epithelium; B: Mucosal entry across the follicle-associated epithelium; C: Tolerance to stress, habituation and replication within mucosal macrophages. *afa*: Operon encoding afimbrial adhesin; CEACAM: Carcinoembryonic antigen-related cell adhesion molecule-6; *dsbA*: Gene encoding bacterial disulfide oxidoreductase; *fimH*: Gene encoding bacterial type-1 fimbrial adhesin; *gadA-C*: Glutamate-dependent acid resistance genes; GP2: Glycoprotein 2 receptor; GP96: Endoplasmic reticulum stress response glycoprotein 96; *hfq*: Gene encoding RNA-binding host factor essential for replication of the bacteriophage Q β ; *htrA*: Gene encoding high temperature stress protein A; IECs: Intestinal epithelial cells; *lpfA*: Gene encoding long polar fimbriae adhesin; M cells: Microfold cells; *ompC*: Gene encoding outer-membrane vesicle protein C; *rpoS/E*: Genes encoding stress tolerance sigma factors.

falling away^[87].

Ileal AIEC isolates also typically express type-1 pili (FimH) on their surface supporting adherence to ileal enterocytes *via* interaction with carcinoembryonic antigen-related cell adhesion molecule-6 (CEACAM6) receptors known to be over expressed on the inflamed ileal (but not colonic) epithelium in Crohn's^[88]. Highly glycosylated CEACAMs have also been proposed as M cell microbial receptors^[89]. It is plausible that one or more members of the CEACAM receptor family may play an important role in regulating endocytosis of CD mucosa-associated *E. coli* into host M cells. A recent study also reported that the glycoprotein 2 (GP2), specifically expressed on the apical plasma membrane of M cells among enterocytes, is recognized by FimH^[90]. By an intriguing coincidence it has also recently been found that the same GP2 protein is the epitope for the "anti-pancreatic" antibody found in CD sera^[91]. In addition, Crohn's AIEC outer-membrane vesicles (OMV), also show ability to interact with enterocyte endoplasmic reticulum stress response glycoprotein 96 receptor, increased in expression on the inflamed intestinal epithelium^[92]. These OMVs, in association with flagellin, also possess significant ability to evoke pro-inflammatory cytokine release^[93]. Colonic

mucosally associated AIEC isolates expressing afimbrial adhesin *afa* operon, more commonly associated with diarrhoeagenic diffusely adherent *E. coli*, have also been observed to be more prevalent in CD patients than in non-IBD controls^[84]. The presence of the *afa* operon correlates with diffuse adherence to, and invasion of intestinal epithelial cells^[84].

A summary of Crohn's AIEC genotype relevant to host intestinal mucosa interactions is summarised in Figure 3.

VIRULENCE FACTORS SUPPORTING CROHN'S AIEC SURVIVAL AND REPLICATION WITHIN HOST MACROPHAGES

AIEC isolated from Crohn's ileal and colonic biopsy tissue demonstrate ability to survive and replicate within phagolysosomes of host macrophages^[94,95]; see Figure 4. However, they are not unique in this ability as other pathogens are also known to survive and replicate within macrophages, including *Mycobacteria*, *Salmonella*, *Shigella*, *Coxiella*, *Brucella*, *Legionella* and *Listeria* species. Key de-

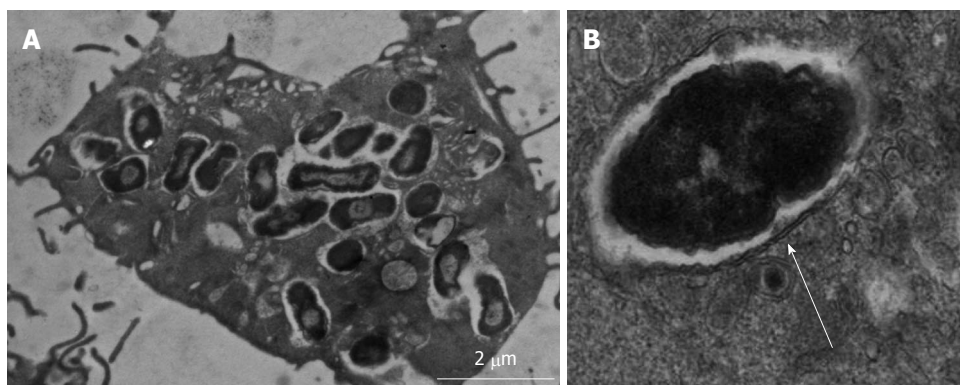


Figure 4 Transmission electron micrograph of adherent, invasive *Escherichia coli* within macrophages¹. A: Crohn's disease colonic mucosa-associated isolate HM605 surviving and replicating within vesicles of J774-A1 murine macrophages; B: Double membrane around intra-macrophage vesicle indicates bacteria are contained within phagolysosomes (arrow). ¹Images courtesy of Dr. Carol L Roberts (University of Liverpool, United Kingdom).

fence mechanisms adopted by these pathogens support their resistance to killing within the low pH, low nutrient environment, high oxidative and nitrosative stress environment of the phagolysosome. For example, *Shigella* and *Listeria* are able to escape from the mature phagolysosome, *Salmonellae* can inhibit fusion of phagosome with the lysosome, whilst *Mycobacterium tuberculosis* is able to modify the intra-phagolysosome environment^[96]. Key genes supporting AIEC survival and replication within macrophages have been identified (see Figure 3) using isogenic mutants of the “paradigm” ileal AIEC LF82, including *htrA* (encoding high temperature stress protein), *dsbA* (encoding an oxidoreductase) and *hfq* (encoding a RNA chaperone important in mediating bacterial adaptation to chemical stress)^[97-99]. However, HtrA and DsbA are fairly ubiquitous in *E. coli*, and it is likely that other unidentified factors are needed to support AIEC survival within the stressful conditions of the phagolysosome.

Acid stress is the antimicrobial environment likely encountered by active enteric bacteria within the phagolysosome. *Salmonella* spp., *Shigella* spp. and *E. coli* have all been reported to possess a repertoire of low pH inducible systems that support resistance, tolerance and habituation during environmental acid stress. Likewise, AIEC certainly appear to be tolerant of the low pH intra-phagolysosome environment^[97]. *E. coli* is notable due to its possession of four known acid resistance systems. The first system requires sigma factor RpoS and the cyclic AMP receptor protein CRP, with RpoS functioning as a major environmental stress response regulator in both *E. coli* and *Salmonellae*^[100]. Deletion of RpoS from a Crohn's AIEC (strain O83:H1) has been observed to increase sensitivity of this clinical isolate to oxidative stress^[101]. The second system requires extracellular glutamate. The components of glutamate-dependent acid response are two isoforms of glutamate decarboxylase encoded by *gadA* and *gadB*, and a glutamate-γ-aminobutyric acid antiporter encoded by *gadC*^[102,103]. Murine AIEC have been observed to respond to chronic intestinal inflammation by up-regulating expression of *gadA* and *gadB*^[104]. The third acid resistance system requires is arginine-dependent utilising of arginine decarboxylase (AdiA and AdiC) an-

tiporter^[100] and the fourth is lysine dependent, involving lysine decarboxylase^[103]. In addition, *E. coli* also harbour specific mechanisms that enable them to resist high levels of ROS that form the oxidative and super-oxidative response to phagocytosed pathogens. These defensive resources have recently been found to be grouped particularly into two regulated sets of genes *saxRS* and *oxyR* regulons^[105,106].

DEFECTIVE AUTOPHAGY AND LACK OF CLEARANCE OF AIEC

ATG16L1 and IRGM function in autophagosome formation and evidence from our own studies supports a role for autophagy as an antimicrobial mechanism downstream of toll-like receptor and NOD-like receptor signalling. Activation of NOD2 by MDP induces autophagy in antigen-presenting cells (such as dendritic cells and macrophages) in a receptor-interacting serine-threonine kinase-2 dependent manner^[107]. Knock-down of *ATG16L1* and *IRGM* using siRNA approaches results in defective recognition and clearance of Crohn's mucosa-associated *E. coli* within host epithelial cells and macrophages^[108]. However, deficiency in either gene did not interfere with the replication and survival ability of other non-pathogenic, environmental, commensal, or gastroenteritis-inducing *E. coli*, suggesting a specific role for autophagy in restraining AIEC. Similarly, expression of the Crohn's variant *ATG16L1**300A in intestinal Caco2 epithelial cells impairs their ability to capture internalized *Salmonella* spp. within autophagosomes^[109] and is also associated with abnormalities in Paneth cell granule exocytosis^[110], impaired production of antimicrobial α-defensins^[111], and increased production of pro-inflammatory cytokines IL-1β and IL-18 by macrophages in response to LPS^[112].

STRATEGIES TO TARGET INTRA-MACROPHAGE AIEC IN CD

If AIEC have a primary pathogenic role then it follows

that targeted treatment should lead to clinical benefit. This hypothesis is supported by studies in Boxer dogs which develop a granulomatous colitis following infection with an AIEC strain^[69], with subsequent clinical resolution following treatment with the 4-quinolone antibiotics, enrofloxacin^[113]. However bacterial antibiotic resistance is common both in animal and human studies and is associated with poor clinical outcome^[114]. Trials of antibiotics in the treatment of active CD have been disappointing to date with good evidence only for their use in the prevention of post-operative disease recurrence^[115,116]. A large metanalysis recently failed to show any clear benefit for their use in maintenance of remission or in the treatment of active luminal or peri-anal disease^[117]. In some trials, early open label studies were positive only for later randomised trials to fail to show clear benefit^[118,119], which may, in part, be due to the development of antibiotic resistance. *In vitro*, quinolone-based antibiotics regimens to target intra-macrophage Crohn's AIEC isolates are effective^[95] but again single antibiotic use likely increases the risk of drug resistance, a problem highlighted by a recent study in which multidrug resistance was seen in 61.5% of Crohn's AIEC isolates^[120]. Triple antibiotic regimens are superior to ciprofloxacin mono-therapy and reduce intra-macrophage AIEC survival to 3% relative to untreated controls^[95]. Unfortunately significant drug-drug interactions occur with some antibiotics and azathioprine which have limited the use of triple combinations to date. Consequently, alternative strategies are being explored including using adjuvant agents to manipulate the phagolysosomal environment to support microbial phagocytosis.

A more promising strategy may be to alter phagolysosomal pH to aid bacterial killing within macrophages. It has already been shown that AIEC are dependent on an acidic environment for survival^[97] and that alkalinisation leads to reduced survival. Hydroxychloroquine, a weak base able to increase phagolysosomal pH, is known to improve killing of bacteria where intra-macrophage survival plays a key step in disease pathogenesis^[119]. For example, *Coxiella burnetii* the agent of Q fever, maintains an intracellular lifestyle through adaptation to survival at an acidic pH^[121,122]. *Coxiella* survival was significantly reduced *in vitro* by hydroxychloroquine treatment and this benefit translated into clinical response in a randomised trial^[123,124]. Hydroxychloroquine in combination with antibiotics, is also now standard therapy for treatment of Whipple's disease, where replication of *Tropheryma whippelii* within tissue macrophages is a central part of the pathogenesis^[125]. Similarly, our own recent studies have shown that dose-dependent enhancement of macrophage killing of Crohn's AIEC can be seen with hydroxychloroquine treatment and synergy with standard antibiotics is also observed^[126].

Vitamin D supplementation also enhances killing of intracellular AIEC in both murine and human macrophages^[127]. This may be due to enhancement of the respiratory burst but effects are likely to be multimodal with influences on several intracellular pathways. Cellular

production of the antimicrobial peptides, such as cathelicidin antimicrobial peptide (CAMP) and $\beta 2$ defensin, follows stimulation of toll-like receptors in the presence of vitamin D and conversely, vitamin D deficiency leads to impaired macrophage function due to defective defensin production^[128]. This has significance in CD, where muramyl dipeptide stimulation in the presence of vitamin D leads to increased CAMP expression. Furthermore, vitamin D stimulates NOD2 expression and leads to downstream $\beta 2$ defensin production^[129]. Vitamin D deficiency is common in CD with up to 70% of patients affected, even in quiescent disease^[130,131]. This now appears to have clinical consequence with several studies demonstrating a correlation between serum levels and disease behaviour. In a large prospective cohort study with nearly 1.5 m patient years of follow up, a validated method for predicting vitamin D levels was used to compare the incidence of CD in the lowest quartile relative to the highest quartile, finding the highest risk associated with the lowest Vitamin D levels^[132]. This correlation is not limited to the relative disease risk and recent studies now show a clear correlation between disease behaviour and serum concentrations. CD activity, defined both by CDAI and CRP level, has been shown to be inversely correlated with Vitamin D levels, with greatest activity seen in those with the lowest levels^[133]. Furthermore, in a retrospective study of 3217 patients, a lower likelihood of requiring surgery for Crohn's was seen with higher vitamin D levels, when using a cut off of 30 ng/mL^[134]. Given these findings we might therefore expect a clinical effect from Vitamin D supplementation. This question was addressed in a randomised double-blind placebo-controlled trial in which a trend was seen towards lower relapse rates in patients treated with 1200 U/d of Vitamin D, although this did not quite reach significance^[135]. However a significant reduction in risk of requiring surgery was seen for deficient patients who normalised their vitamin D levels with supplementation^[134]. Overall these data suggest a clinical role for vitamin D supplementation in CD although further clinical trials are required. Whilst no data yet exists for the effect of vitamin D on AIEC-macrophage interactions *in vivo*, it appears that supplementation may hold promise as a clinical strategy for targeting Crohn's mucosa-associated *E. coli*.

Smoking has long been associated with disease activity and leads to greater treatment requirements, more stricturing disease, more peri-anal disease and shorter disease free survival^[135,136]. These affects are likely to be multimodal in origin with effects seen on macrophage function, gut microbiota and vitamin D levels^[137-139]. Interventional studies clearly show benefit from smoking cessation^[140] and that this is an achievable therapeutic aim^[141]. There are some data to support a hypothesis that this may in part be due to recovery of immune cell function but to date this has not been systematically studied in CD^[142].

CONCLUSION

Based on the findings of a diversity of individual studies,

there has been accumulating evidence proving the implication of bacteria such as AIEC in the pathogenesis of CD, a chronic-relapsing IBD. AIEC have been shown to translocate M cells of Peyer's patches and lymphoid follicles of the colon, and then to survive and replicate within underlying mucosal macrophages and dendritic cells. However, the mechanism of how Crohn's AIEC resist killing process and adapt to the environment within the phagolysosome to survive and grow within macrophages without inducing cell death is still poorly understood. There is no doubt that further investigation is warranted to characterise and identify the key virulence factors relevant to AIEC phenotype, supporting current and novel, targeted treatments for future clinical benefit.

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Neurological disorders and inflammatory bowel diseases

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Key words: Inflammatory bowel diseases; Crohn's disease; Ulcerative colitis; Extraintestinal manifestations; Neurological disorders; Multiple sclerosis; Progressive multifocal encephalopathy; Demyelinating neuropathies; Cerebrovascular diseases; Side effects

Core tip: Extraintestinal manifestations occur in about one-third of patients with inflammatory bowel disease (IBD) and may precede the onset of gastrointestinal symptoms by many years. Neurological disorders are uncommon in IBD but they can represent an important cause of morbidity and relevant diagnostic issue. Furthermore, the use of immunosuppressant and biological therapies for IBD may also play a pivotal role in the development of neurological disorders. Hence, we review the main features of neurological complications associated with IBD, with particular reference to those related to drugs, thereby focusing on their clinical presentation and possible pathophysiological mechanisms.

Abstract

Extraintestinal manifestations occur in about one-third of patients living with inflammatory bowel disease (IBD) and may precede the onset of gastrointestinal symptoms by many years. Neurologic disorders associated with IBD are not frequent, being reported in 3% of patients, but they often represent an important cause of morbidity and a relevant diagnostic issue. In addition, the increasing use of immunosuppressant and biological therapies for IBD may also play a pivotal role in the development of neurological disorders of different type and pathogenesis. Hence, we provide a complete and profound review of the main features of neurological complications associated with IBD, with particular reference to those related to drugs and with a specific focus on their clinical presentation and possible pathophysiological mechanisms.

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INTRODUCTION

Inflammatory bowel diseases (IBDs) are common causes of gastrointestinal morbidity in western countries. Extraintestinal manifestations are frequent in the course of IBD and, in some cases, may be the first manifestation of IBD, sometimes preceding the onset of gastrointestinal symptoms by many years^[1].

Among the many extraintestinal manifestations af-

fecting various organs, neurological disorders of different type and pathogenesis have been documented^[2]. Overall, the occurrence of neurological disorders during the course of IBD is uncommon^[3], but they may represent an important cause of morbidity. Neurological complications appear to be more common in men and they usually appear after IBD diagnosis, rarely coinciding with exacerbations of the underlying bowel disease^[3]. Only a few systematic studies have investigated their frequency in patients with IBD and the results have been frequently inconsistent due to differences in the methods used for case finding and outcome evaluation^[4].

One of the largest studies, performed for this purpose on 638 patients with IBD [either ulcerative colitis (UC) or Crohn's disease (CD)], found neurological disorders in 3%^[3], with another study^[5] indicating a possible increase in the prevalence of demyelinating diseases, particularly of multiple sclerosis (MS). The increasing use of immunosuppressant and biological therapies may also influence the probability of IBD-associated neurological disorders because these agents, although rarely used, may cause central nervous system (CNS) white matter lesions^[6], opportunistic infections^[7] with clinical symptoms similar to MS^[2], or JC virus (JCV)-mediated progressive multifocal leukoencephalopathy (PML)^[7].

On the basis of these considerations, it is obvious that a physician treating IBD/patients should be able to recognize unexplained neurological symptoms, consider their association with IBD, and address a proper diagnostic and therapeutic work-up, possibly with the collaboration of a consultant neurologist.

In this review, we went through the different neurological complications associated with IBD, with particular reference to those related to drugs, with a specific focus on their clinical presentation and their possible pathophysiological mechanisms^[8]. We divided the review into the following specific sections: (1) side effects of medications; (2) cerebrovascular diseases; (3) immune-mediated neurological disorders; and (4) miscellaneous.

SIDE EFFECTS OF MEDICATIONS

Biologics

Anti-tumor necrosis factor (TNF)- α drugs such as infliximab, adalimumab, certolizumab, etanercept, oncept^[9] and anti- α 4 integrin such as natalizumab and MLN020^[10], generally referred to as biologic drugs, have all been tested in the treatment of IBD, but etanercept, oncept and MLN020 have not been registered for clinical use, and thus have only had a limited exposure. Although their use has been occasionally associated with the induction or exacerbation of several neurological diseases in IBD patients^[11], the diagnosis of a possible causal relationship is usually made on the time-correlation between the use of the drug and the appearance of the neurological manifestation^[12]. In a screening study by the Food and Drug Administration on Adverse Event Reporting System (FAERS), Deepak *et al*^[13] reported 772 distinct neurological adverse effects secondary to TNF- α inhibitor

exposure over a 10-year period. Thus, particular attention should be placed when an IBD patient on biologic therapy develops neurological symptoms, looking for a cause-effect relationship^[14]. Conversely, in patients with neurological diseases before the start of biologic therapy, a neurological consultation must be performed and possible alternative therapies, including surgery, should be considered.

PML

Because of its severity and high mortality rate, PML is the most feared neurological complication for patients treated with biologics. Although its occurrence was originally observed in patients treated with natalizumab in combination with β 1 α interferon for MS, this disease has also been reported in IBD patients treated with natalizumab only and also in very few cases undergoing anti-TNF therapy^[7]. PML is a demyelinating disease caused by the reactivation of JCV, a virus with specific tropism for glial cells in the brain^[15]. JCV may be reactivated from sites of latency in lymphoid tissues in conditions characterized by reduced immune surveillance^[16]. The explanation for the appearance of PML in natalizumab-treated patients is apparently related to both the action of JCV and to a probable increased release of infected lymphocytes from bone marrow determined by the binding of α 4 β 1 integrin with the drug^[17].

Yousry *et al*^[18], in a review of about 3000 patients treated with natalizumab for MS, CD or rheumatoid arthritis, did not find any case of PML, thus suggesting a risk of PML of < 1 per 1000 patients treated for a mean time of 17.9 mo. The drug, at this moment, is not approved for IBD in Europe, while its use is confined to patients showing no response to anti-TNF agents in the United States. Clinicians should consider PML in the presence of visual defects (45% of all cases) and/or mental impairment (38% of all cases) such as dementia, confusion and personality changes. Indeed, cognitive impairment and behavioral changes frequently are the earliest clinical manifestations of PML^[14]. A motor weakness may also be present.

The diagnosis is confirmed by magnetic resonance imaging (MRI), which reveals white matter lesions with typical T2 and T1 signals^[18]. Cerebrospinal fluid (CSF) examination is usually normal but polymerase chain reaction (PCR) amplification of the JCV DNA is an important diagnostic tool. It is debated whether patients with IBD, similarly to what happens for MS patients treated with natalizumab, should undergo serial testing for anti-JCV antibodies before and during treatment either with natalizumab or with anti-TNF^[18]. Indeed, patients on natalizumab can be risk stratified for the development of PML based on JCV antibody status, history of immunosuppressive drug and duration of natalizumab treatment. Singh *et al*^[14] described that all cases of natalizumab-induced PML occurred in patients who were JCV antibody positive. The seroprevalence of JCV-specific IgG in healthy blood donors is estimated to be 50% by 30 years of age and this percentage in-

creases to 60% by 70 years of age^[14]. The risk increases with duration of natalizumab therapy, particularly after 24 mo, but some cases have been reported after only 6 mo of therapy^[14].

Discontinuation of natalizumab is recommended at the first suspicion of PML^[14], and plasmapheresis is the recommended therapy to remove natalizumab, accelerate desaturation of the targeted $\alpha 4$ -integrin receptors and restore leukocyte transmigration^[14]. When immunosuppression is rapidly reverted in cases of natalizumab-associated PML, an exuberant immune response may occur: this condition has been termed immune reconstitution inflammatory syndrome^[14]. The response targeting JCV is evident 2–6 wk later in the CNS and it often results in paradoxical worsening of PML symptoms^[14]. High dose corticosteroids are recommended if clinical and radiographic worsening are noted several weeks after immune restoration. Despite these treatments, the clinical outcome of natalizumab-induced PML patients is poor with a reported mortality of 60% in patients with 6 mo follow-up^[14].

Posterior reversible encephalopathy syndrome and similar diseases

Zamvar *et al*^[19] described a posterior reversible encephalopathy syndrome in a 14-year-old boy affected by CD following infliximab infusion with generalized tonic-clonic seizures and visual disturbances probably caused by occipital lobe involvement. The patient recovered after drug discontinuation by which time he returned to normal.

Brigo *et al*^[20] also described a 74-year-old man with CD, without a prior history of seizures, presenting with a seizure after the second infusion of infliximab and caused by a reversible encephalopathy syndrome, an acute form of encephalopathy characterized by headache, seizures and area of increased T2 signal in the posterior quadrants of the brain on MRI^[20]. A direct correlation between seizures and infliximab treatment is likely in these cases because a sharp clinical improvement occurred 7 d after infliximab discontinuation.

Faivre *et al*^[21] described a 64-year-old woman with CD developing encephalitis associated with acute neuropathy after two infusions of infliximab. The patient had acute anterograde memory deficiency associated with epileptic episodes without infectious, vascular, tumor or toxic causes^[21]. MRI showed bilateral hippocampal hypersignals suggestive of limbic encephalitis. The clinical symptoms disappeared after infliximab withdrawal and seizures never relapsed even after discontinuation of antiepileptic drugs.

CNS vasculitis

The most common autoimmune manifestation associated with anti-TNF therapy is the development of anti-nuclear antibodies and anti ds-DNA autoantibodies without an associated clinical syndrome^[22]. A systemic lupus erythematosus (SLE)/lupus-like syndrome occurs in some of these patients and it has been named anti-

TNF-induced lupus (ATIL). This syndrome^[23] usually shows a high prevalence of anti-dsDNA antibodies (> 90%) and a low prevalence of anti-histone antibodies (57%) in contrast to what is usually seen in drug-related lupus syndromes. As in patients with spontaneous lupus, patients with ATIL may develop vasculitis. This was the case in a 53-year-old woman with ileo-colonic CD in whom adalimumab therapy was complicated by the development of SLE with CNS vasculitis. The patient showed headache, drowsiness, visual defect in her right eye associated with pleural, peritoneal and pericardial effusion 4 mo after initiation of adalimumab therapy at a dose of 40 mg subcutaneously every other week. Brain MRI showed features suggestive of cerebral vasculitis.

Ramos-Casals *et al*^[24] found 233 cases of autoimmune diseases possibly induced by TNF-targeted therapies with a prevalence of vasculitis and lupus respectively of 48% and 39%. Among the 92 patients with ATIL, CNS vasculitis was observed only in two patients (1 treated with infliximab for CD and 1 treated with etanercept for rheumatoid arthritis). In the case reported by Vannucchi *et al*^[22], the autoantibodies disappeared and the clinical picture returned to normal 6 mo after anti-TNF withdrawal.

MS

The development or exacerbations of MS^[25] or CNS demyelination are well-described neurological complications of TNF- α antagonist therapy. TNF- α antagonist might be effective for inflammatory neurological disorders such as MS because elevated levels of TNF- α have been demonstrated in serum or CSF of patients with MS^[26].

Microglia and macrophages in the CNS secrete TNF- α with a direct role in the pathogenesis and demyelination of MS^[27]. There are two forms of TNF- α : a trans-membrane protein (tmTNF) and a soluble form (sTNF); both interact with two distinct receptors, TNFR1 and TNFR2^[28]. In the first stages of MS, TNF- α is involved in demyelination, while in later stages it is fundamental for remyelination^[29]. In a double-blind placebo-controlled phase II human study of lenercept, a recombinant TNFR1 fusion protein, more lenercept-treated patients experienced exacerbations compared to placebo patients and these exacerbations occurred early, leading to early study termination^[30].

There are several case reports of the development of MS^[31] or CNS demyelination during treatment with TNF- α antagonist such as infliximab or adalimumab. The mechanisms of induction or exacerbation of MS and/or CNS demyelination are unknown. One possible hypothesis is that TNF- α has anti-inflammatory effects that may contribute to “off” signals in MS. The “on/off” balance of TNF-mediated signals is relevant to MS and the removal of TNF- α might potentiate the disease^[30]. Anti TNF- α drugs, particularly infliximab, do not appear to cross the blood-brain barrier and neutralize local TNF- α -mediated tissue injury. Nevertheless, infliximab causes enhanced permeability of the barrier increasing the activation of myelin-specific peripheral autoreactive T cells^[32]. TNFR2 function is important for the enhance-

ment of remyelination and the use of TNF- α antagonists may inhibit the tmTNF-TNFR2 axis^[33].

The Mayo Clinic reported one case of MS in 500 CD patients treated with infliximab and another patient has been reported among 651 IBD patients treated with infliximab in the Danish Crohn Colitis database; three further cases of MS were reported in Edinburgh's experience of 620 patients treated with IFX^[34]. It is unclear whether these demyelinating events are coincidental or causally associated with the use of TNF- α antagonists because the interval between the administration of anti-TNF- α agents and the appearance of symptoms varies greatly. Most researchers have reported that the average time between the beginning of treatment and the onset of neurological symptoms is about 5 mo^[35].

Demyelinating neuropathies

The proposed pathogenesis of anti-TNF α -associated neuropathies encompasses both T cells and humoral immune attacks against peripheral nerve myelin, vasculitis-induced nerve ischemia and inhibition of signaling support for axons^[11]. Most of these neuropathies improve over a period of several months after withdrawal of the drug, with or without additional immunomodulating treatment^[11].

Guillan-Barré syndrome and its variant Miller-Fisher syndrome

Guillan-Barré syndrome (GBS)^[36] and Miller-Fisher syndrome^[37] are two types of demyelinating peripheral neuropathies reported during treatment with TNF- α antagonists. GBS is a post-infectious, immune-mediated disease, generally presenting as an acute inflammatory demyelinating polyneuropathy^[36] characterized by ascending paralysis with rapid, progressive, symmetric limb weakness and areflexia.

The annual incidence is 1.5 cases/100000 and the mortality rate is about 5%. Approximately 10% of patients are still severely disabled at 1 year after diagnosis. Miller-Fisher syndrome is a rare variant of it and its main manifestation is descending paralysis affecting the eye muscles with the triad ophthalmoplegia, ataxia and areflexia^[37]. It is possible that TNF- α antibodies unmask latent infections or cause an increased susceptibility to infections triggering or worsening the autoimmune demyelinating processes^[11]. Two-thirds of GBS cases are associated with bacterial or viral infections such as *Campylobacter jejuni*, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and varicella zoster virus^[37].

It is important to remember that GBS may be an independent extraintestinal manifestation of IBD induced by vasculitis, malnutrition or vitamin deficiencies^[37]. Drug withdrawal is always suggested in the management of these patients and, in troublesome cases, cyclophosphamide or intravenous immunoglobulin is needed^[37]. Vadikolias *et al*^[38] reported a case of a 40-year-old man affected by CD developing autoimmune demyelinating

acute paraplegia 4 mo after starting infliximab therapy. Some authors suggest that search subclinical demyelinating processes before initiating anti-TNF- α therapy, particularly in young patients^[5], should become a recommended standard practice. Shin *et al*^[36] described 15 cases of GBS identified from the postmarketing database of anti-TNF (9 patients on infliximab, 5 on etanercept, and 1 on adalimumab). The symptoms were reported between 6 wk and 2 years after the start of these therapies^[36]. Thirteen patients have been subjected to regular follow-up and 12 showed a partial or complete resolution after adequate therapy for GBS^[36]. Deepak *et al*^[13], in the FAERS about neurological events in patients treated with TNF- α inhibitors, reported 153 cases out of a total of 772 with neurological manifestations related to anti-TNF- α therapy.

Lewis-Summer syndrome

Nancey *et al*^[39] reported two cases of Lewis-Summer syndrome (LSS), also called multifocal acquired demyelinating sensory and motor neuropathy, related to the use of infliximab. LSS is a rare, dysimmune, multifocal peripheral nerve disorder described for the first time in 1982 by Lewis *et al*^[40], and characterized by asymmetric multifocal and sensory involvement of the nerve roots and trunks of the upper and lower limbs. The disease should be suspected in the presence of distal, asymmetric weakness affecting the upper or lower limbs with initial sensory impairment followed by motor involvement^[39]. An electromyographic study showing persistent multifocal conduction blocks allows the diagnosis^[39]. LSS involves only peripheral nerves without any damage to central myelin^[39].

Multifocal motor neuropathy with conduction block

Barber *et al*^[41] reported one case of multifocal motor neuropathy with conduction block (MMNCB) following treatment with infliximab. This is an asymmetric motor neuropathy with diagnostic features including the presence of multifocal partial motor conduction block and the presence of blood anti-GM1 antibodies in 50% of cases. No more than 10 cases of MMNCB associated with anti-TNF therapy have been reported in the international literature. The rate of progression of demyelinating neuropathies is highly variable ranging from a few days to many years and the recovery is not always certain after drug withdrawal^[41].

Following suspicion of peripheral neuropathies related to biologic therapy, a consultation with a neurologist should always be suggested for a differential diagnosis between small fiber polyneuropathies and an axonal sensory-motor neuropathies (SM-PNs) characterized by areflexia, sensory ataxia, minor cutaneous sensory deficiency (distal dysesthesia)^[41] and variable degree of motor dysfunction.

Ischemia and inhibition of signaling support for axonal transport are the mechanisms proposed for secondary axonal loss. It is conceivable that the adverse effect

of TNF- α antagonists on peripheral nerves is cumulative and, therefore, the severity of neuropathy is proportional to the total dose of the drug received^[3]. These data emphasize the importance of long-term vigilance during the course of the treatment with TNF- α antagonists. In most cases, drug withdrawal resolves the complication^[3].

Chronic inflammatory demyelinating polyneuropathy

Some cases of Chronic inflammatory demyelinating polyneuropathy (CIDP) have been reported during anti-TNF therapy^[42,43], appearing 4-17 mo after initiation of infliximab, with the possible presence of GM2 antibodies in the serum. CIDP is characterized by weakness in the proximal and distal extremity muscle groups associated with bilateral foot drop and a stocking-glove pattern mostly localized in the lower extremities^[42]. Electrodiagnostic studies reveal progressive acquired demyelinating sensory and motor peripheral polyneuropathy. Withdrawal of the offending agent does not always reverse the immune process and chronic immunotherapy may be needed to control the inflammatory process and improve clinical outcome^[42].

Infections of the nervous system

B-lymphocyte depletion in immunocompromised patients causes meningitis by encapsulated bacterial pathogens, while T-lymphocyte depletion or impaired macrophage function cause the development of infections by intracellular pathogens such as fungi, particularly *Aspergillus* and *Nocardia*, viruses such as herpes simplex virus (HSV), JCV, CMV, human herpes virus 6 and parasites such as *Toxoplasma gondii* (*T. gondii*)^[44].

In general, although anti-TNF agents may increase the risk of infections, even at neurological levels, and particularly for intracellular organisms, several observations indicate that the risk of opportunistic infections is greatly increased when patients are treated with more than one immunosuppressant drug. Thus, the main recommendation originating from these observations is to limit multiple immune suppression to the shortest time possible.

Patients with infections of the nervous system may present with many clinical manifestations including meningeal signs, mass lesions, encephalopathy, seizures and stroke-like presentation^[44].

CNS fungal infections

CNS infections by *Aspergillus* are usually characterized by mass lesions such as brain abscesses or by cerebral infarction, and more rarely by meningitis^[44]. *Cryptococcus neoformans* may show as subacute meningitis with fever and headache without neck stiffness. Mass cerebral lesions usually have a subacute or chronic presentation while meningitis and encephalitis have a more acute presentation. In patients with meningeal signs and/or encephalopathy, a lumbar puncture should be performed with strain culture or serology of CSF^[44]. MRI should always be performed in patients with a strong clinical suspicion

of encephalopathy. MRI should be performed and tissue biopsy sampling may be always considered^[44] in patients with cerebral mass lesions, for a differential diagnosis between tuberculosis, lymphoma and toxoplasmosis. Lumbar puncture should also be always performed in these cases because a positive EBV PCR in the CSF suggests the presence of CNS lymphoma.

Meningococcal meningoencephalitis

Majumder and Kumar^[45] described a case of a 51-year-old woman affected by CD showing meningococcal meningitis during treatment with certolizumab in clinical remission for 6 mo. Meningococcal vaccination is safe and it should always be considered in high-risk IBD patients, particularly in those subjected to biologic therapy^[46].

Listeria infection

In 2000 Morelli *et al.*^[47] described for the first time a *Listeria* infection complicating infliximab therapy in a CD patient. *Listeria monocytogenes* (*L. monocytogenes*) is a Gram-positive, rod-shaped, facultative intracellular organism. About 1%-5% of all healthy adults are asymptomatic carriers of *L. monocytogenes*. In the United States, 2500 cases/year are reported and the mortality rate is 15%-30%. Mortality of *Listeria* meningoencephalitis infection, associated with sepsis, is particularly high in pregnant women, neonates and immunocompromised subjects, where it may reach 33% of cases^[47]. Listeriosis is a foodborne infection caused by ingestion of soft cheeses, unpasteurized milk, unwashed vegetables, ready-to-eat foods such as hot dogs and cold cuts, and it may be more frequent in populations eating raw food such as those living in Africa and Asia. It is important to wash the hands and to scrub fruits and vegetables^[47]. TNF- α , produced by monocytes, macrophages, lymphocytes, and fibroblasts, has a crucial role against *L. monocytogenes* as well as all other intracellular organisms. In fact, TNF- α is crucial in host resistance against intracellular organisms by mediating local inflammation to control infection^[47]. Generally, blood culture becomes positive for Gram-positive bacilli after 32 h and lumbar puncture reveals a high percentage of polymorphonuclear leukocytes. Ampicillin and gentamicin is the treatment of choice for listeriosis^[47]. On the basis of the above considerations, it is important that patients receiving anti-TNF therapy observe safe food practices and refrain from the above-mentioned food products.

Campylobacter fetus infection

Umehara *et al.*^[48] described *Campylobacter fetus* (*C. fetus*) meningitis in a CD patient on long-term steroid therapy after only one infusion of infliximab. *C. fetus* is a Gram-negative, motile, bacterial species with a typical S-shaped rod morphology and it is particularly present in immunocompromised, pregnant women and neonates in whom it may cause endocarditis, thrombophlebitis, pneumonia, pleurisy and arthritis^[49]. The bacterium lives in the intestinal flora of cattle and sheep where it causes

spontaneous abortions^[50].

Toxoplasmosis

Young *et al*^[51] described a case of cerebral toxoplasmosis during infliximab therapy associated with low doses of prednisone, methotrexate and leflunomide in a 36-year-old woman affected by severe rheumatoid arthritis. The most important neurological symptoms were diffuse headache, slurred speech, weakness in the left arm, and a grand mal seizure. MRI imaging showed two lesions within the right hemisphere and the diagnosis of *T. gondii* infection was obtained by brain biopsy^[51]. *T. gondii* is an obligate intracellular parasite infecting up to a third of the world's population^[51]. *T. gondii* infection is acquired by ingestion of food or water contaminated with oocysts shed by cats, or by eating undercooked or raw meat containing tissue cysts^[52]. Primary infection is usually subclinical but, in some patients, cervical lymphadenopathy, ocular disease, encephalitis, myocarditis and pneumonitis may occur^[52]. The disease may be life-threatening in immunocompromised patients, and CNS involvement has been described in AIDS patients^[52], and patients receiving corticosteroid^[52] and anti-TNF- α ^[52] therapy. Lassoued *et al*^[53] described two cases of chorioretinitis related to *T. gondii* during anti-TNF therapy, with malaise, low-grade fever and visual defects as major complaints^[52]. TNF- α has an important role in the protection against *T. gondii* infection, playing a synergic role with interferon γ ^[54]. Response to treatment for toxoplasmosis occurs early and, in patients with compatible MRI of the brain, empirical treatment should be started^[51]; early response to treatment usually confirms toxoplasmosis diagnosis.

Nocardiosis

Wendling *et al*^[55] described cerebral nocardiosis during adalimumab and methotrexate therapy for rheumatoid arthritis. A 63-year-old Caucasian man showed the appearance of subcutaneous nodules in the trunk with histological diagnosis of pyogenic granulomas, pulmonary nodules upon chest radiography, and neurological signs such as headache, vertigo, cerebellar dysarthria after 8 mo combined therapy^[55]. Brain computed tomography (CT) and MRI showed two lesions with edema and a mass effect in the right parietal region and cerebellum. Surgical biopsies revealed a pyogenic abscess and the presence of *Nocardia farcinica*. Nocardiosis is caused by an opportunistic, aerobic, Gram-positive, filamentous bacterium of the order Actinomycetales. The most common species are *Nocardia asteroides*, *Nocardia brasiliensis*, and *Nocardia otitidis cavatum*. This bacterium has a long incubation period^[55]. Modes of contamination include inhalation and direct inoculation through the skin^[55]. Systemic nocardiosis is defined by the presence of two or more foci of infection^[55]; the lung is the most common primary site of systemic nocardiosis (60%-80% of cases) and cerebral or other locations may occur in 20%-40% of cases. CNS involvement is responsible for the worst

prognosis with a 75% rate of mortality, particularly in lupus patients^[53]. TNF plays a role in the clearance of *Nocardia* in animal models^[55]. However, nocardiosis is rare during anti-TNF- α therapy and only eight cases^[55] have been reported among 300000 patients treated with anti-TNF agents in the United States. Anti-TNF- α may accelerate and disseminate previously undiagnosed nocardiosis, particularly when therapy comprises corticosteroids and methotrexate.

Herpes simplex infection

Herpes simplex encephalitis (HSE) has been reported in patients receiving TNF- α antagonist therapy^[56]. Also, TNF- α inhibitor therapy appears to be associated with an increased risk of herpes zoster^[57]. An increase in the risk of severe HSV infection is related to the use of TNF- α inhibitors because TNF is an important element of the innate immune response to HSV-1 encephalitis, as reported in animal models^[58]. About 95% of all cases of HSE are attributed to HSV-1 and, only occasionally, HSV-2 has been described^[59]. Bradford *et al*^[56] has identified three adults affected by HSE during monoclonal antibody TNF- α inhibitors. The patients clinically showed altered mental status, such depression or reduced affective response, slow mental processing, memory disturbances, fever, meningismus and headache. In patients receiving TNF- α the clinical manifestations may be atypical. Brain MRI shows characteristic temporal lobe involvement, CSF PCR positive for HSV DNA, and the clinical picture significantly improves after acyclovir therapy^[56]. More than 90% of adult patients with HSE have temporal lobe abnormalities on brain MRI and a positive HSV PCR at clinical presentation^[60]. Weil *et al*^[61] reported that these diagnostic tests might be initially negative in 5%-27% of patients if they are performed early in the disease course. Bradford *et al*^[56] reported that two of three patients initially had normal brain MRI and negative results for CSF HSV PCR. Recently published guidelines^[62] emphasize the need to repeat HSV PCR in 3-7 d if the results are initially negative and the clinical course is highly suggestive for HSE. Bradford *et al*^[56] suggest that in patients on anti TNF- α therapy and a clinical presentation suggestive for HSE, empirical acyclovir treatment should be performed until the result of a second PCR. The mortality of HSE at 1 year is 14%-22% and most survivors have residual neurological and cognitive deficits^[63].

Lu *et al*^[64] reported a case of Bell's palsy caused by HSV in a 43-year-old woman on adalimumab therapy (40 mg biweekly) for CD for 3 years. The patient showed small painful erythematous ulcers on her oral mucosa and lips, fever and right-sided facial palsy suggestive of Bell's palsy secondary to HSV infection. Her symptoms disappeared after 7 d treatment with three 1-g tablets/d valacyclovir, associated with adalimumab withdrawal, but symptoms recurred after rechallenge with adalimumab. Bell's palsy is an idiopathic peripheral facial nerve paralysis and reactivation of HSV may play a major role

through inflammation of the facial nerve^[65].

EBV infection

Nozaki *et al.*^[66] reported one case of Epstein-Barr encephalitis during TNF- α antagonist therapy but the patient also had concurrent HIV infection. Several studies have investigated the possibility of EBV reactivation in patients treated with infliximab but no significant increased risk emerged^[67]. Lavagna *et al.*^[68], in a study of 60 patients with CD treated with infliximab, did not observe any EBV viremia or any clinical manifestations of EBV infection during and after treatment. Serum EBV DNA was never found in a series of EBV-IgG-positive patients treated with TNF- α blockers^[69].

Cerebral tuberculosis

Tissot *et al.*^[70] described a 40-year-old man with CD treated with infliximab monotherapy for 16 mo complicated by the appearance of neurological symptoms such as blurred vision, and motor and sensitive deficiency of the right lower limb. Clinical examination showed a papular erythematous skin lesion localized on the left lateral cervical region and multiple cervical nodes. Cerebral MRI revealed multiple ring-enhancing lesions suggestive of tuberculoma, and chest-abdomen CT showed an upper lobes alveolar syndrome and multiple abdominal lymph nodes suggestive of miliary tuberculosis. This diagnosis was confirmed by skin lesion biopsy with evidence of giant cell granuloma, and PCR for *Mycobacterium tuberculosis* in cultures of bronchial washing lavage. The patient was subjected to classical anti-tuberculosis treatment, with progressive disappearance of all cerebral lesions and complete resolution of neurological symptoms. Therapy for cerebral tuberculosis should comprise four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) for 2 mo followed by isoniazid and rifampicin for at least 10 mo^[70]. Andrisani *et al.*^[71], in their experience of 92 IBD patients who were candidates for anti-TNF therapy, suggested screening for high-risk latent tuberculosis reactivation during therapy by means of Quantiferon TB-Gold (QFT-G) and tuberculin skin test (TST) because both are useful for identification of high-risk patients.

Ocular nervous disorders

Anterior optic neuropathy^[72] and retrobulbar demyelinating optic neuropathy^[73] have also been reported as possible complications of infliximab therapy. The clinician should be alert to anterior optic neuropathy when patients, during anti-TNF therapy, develop bilateral simultaneous sudden visual loss with decreased visual acuity, without pain related to eye movements, and swollen optic discs with bilateral inferior arcuate defects upon ophthalmology evaluation. Retrobulbar (posterior) optic neuropathy shows the same symptoms of anterior optic neuropathy, perhaps with monolateral visual loss, and only an ophthalmologist may obtain the diagnosis with specific tests such as visual field and flash visual evoked potentials^[73]. Risk factors such as stroke, arterial

hypertension, diabetes, atherosclerosis and hypercholesterolemia should always be ruled out^[74]. Anterior optic neuropathy generally appears early within the first three infusions of infliximab^[74]. In the study of Tissot *et al.*^[70], three patients with infliximab-related anterior optic neuropathy were described; one had impaired visual loss after rechallenge with infliximab infusion, confirming the association between infliximab and optic nerve damage. To date, four cases of toxic, infliximab-related anterior optic neuropathy and 10 cases of retrobulbar optic neuritis have been described^[75]. No patients with toxic anterior optic neuropathy improved after pulsed intravenous infusion of methylprednisolone while all 10 patients with retrobulbar optic neuritis did improve^[73]. At this moment, more data are needed to evaluate the exact pathogenic mechanism and dose relationships between these ocular manifestations and infliximab^[74]. Deepak *et al.*^[13], in a FAERS Study about neurological manifestations in patient on anti TNF- α therapy, reported 105 cases of optic neuritis (13.6%) in 772 patients with neurological manifestations during anti TNF- α therapy.

Steroids

Repeated or prolonged exposure to steroids may cause myopathy that needs to be differentiated from predominant involvement of large motor fibers^[75]. Steroid therapy may also produce a psychotic condition^[76]. Corticosteroid therapy increases the risk of infection in a dose-dependent fashion^[77] and corticosteroid-treated patients with intestinal disease have a relative risk of lethal and nonlethal infections of 1.4 (95%CI: 1.1-1.7, $P = 0.02$). Doses of prednisone > 20 mg/d are associated with a twofold increase in overall relative risk of lethal or nonlethal infectious complications compared with controls ($P < 0.004$). *L. monocytogenes* sepsis and meningitis have been described in adult and pediatric patients treated with high doses of steroids with or without azathioprine^[78]. The long-term use of steroids may also predispose to the development of *C. fetus* meningitis^[78].

Sulfasalazine

Sulfasalazine is still used for both CD and UC because of its low cost/effectiveness ratio. However, its use is mostly hampered by the frequent occurrence of side effects and neurological complications have also been reported.

Severe neurotoxicity leading to drug withdrawal has been reported in $< 5\%$ of patients^[79]. The neurological toxicity of the drug appears mainly to be related to folate deficiency^[80]; a typical adverse result of chronic sulfasalazine intake through different mechanisms: oxidative damage to red cells leading to hemolysis^[81]; inhibition of jejunal hydrolysis of pteroylpolyglutamates blocking absorption of dietary folates; and competition with the three enzymes (dehydrofolate reductase, serine transhydroxymethylase and methylene tetrahydrofolate reductase) mainly involved in folate metabolism. Patients with IBD are predisposed to hyperhomocysteinemia^[80],

which is considered a risk factor for cardio-cerebrovascular events, and part of this condition might be related to the folate-depleting role of sulfasalazine.

Mechanisms other than folate deficiency appear to be present in patients treated with sulfasalazine who develop neurological disorders but the pathophysiological aspects are currently unknown^[81]. Liedorp *et al*^[82] described axonal polyneuropathy occurring after 2 years treatment with sulfasalazine without blood folic acid deficiency. Mut *et al*^[83] noted a reversible encephalopathy after only 3 wk of sulfasalazine therapy, and the clinical symptoms and MRI lesions resolved completely after drug discontinuation. Gold *et al*^[84] have suggested that the drug might also be implicated in the occurrence of MS.

Methotrexate

Methotrexate is an immunosuppressant with anti-folate activity^[85]. It is highly ionized with low lipid solubility and it does not readily cross the blood-brain barrier. Methotrexate is a cell-cycle-specific agent that inhibits the enzyme dihydrofolate reductase, preventing the conversion of folic acid to tetrahydrofolic acid and thus inhibiting cell replication^[85]. Methotrexate also causes a relative excess of homocysteine determining small-vessel vasculopathy^[85]. The risk of neurotoxicity increases with higher doses.

The association between low oral weekly doses of methotrexate and the development of posterior reversible encephalopathy syndrome (PRES) has been described in only a few cases. PRES often shows nonspecific symptoms such as headache, seizures, visual disturbances including cortical blindness, altered mental status, and even coma. Seizures are present in up to 88%, visual disturbances in 60%, and headache with altered mental function in > 50% of patients. In these studies, oral methotrexate had been taken for 3-7 years before the onset of symptoms. The patient described by Hart *et al*^[85] was exposed to a total dose of 1560 mg over 4 years. Thus, the association of neurotoxicity with long-term use of methotrexate suggests a cumulative toxic effect on the blood-brain barrier. Neuroimaging, particularly MRI, is essential to obtain a diagnosis: classical lesions are symmetrical and located in the subcortical and cortical areas of the posterior circulation. The frontal lobe, brainstem, basal ganglia, thalamus, and even the spinal cord may also be involved.

Methotrexate-associated neurotoxicity is often termed as leukoencephalopathy (LEP), frequently presenting as transient seizures^[85]. Also, headache, confusion and disorientation may be present^[86]. LEP is a structural alteration of cerebral white matter in which myelin suffers the most damage. The basic pathophysiological mechanisms leading to methotrexate-induced LEP are unknown but they are multifactorial and include adenosine accumulation, homocysteine elevation, and its excitatory effect on N-methyl-D-aspartate receptor and alteration in bipterin metabolism^[85]. The white matter changes are strictly

localized to the cerebellum and this selective site may be explained by the involvement of Purkinje cell axons^[81]. Only a few methotrexate-induced white matter changes during oral treatment^[87] have been reported and most of them appeared to be related to folic acid deficiency. Chronic folic acid supplementation is thus mandatory in patients undergoing methotrexate treatment to avoid, if possible, these neurological side effects.

Metronidazole

Metronidazole is a commonly used antibiotic in the treatment of IBD, particularly in patients affected by CD^[88] with perianal involvement. Peripheral neuropathy is a well-documented side effect of the drug, reported in 21-39% of CD patients treated with metronidazole, especially in patients receiving > 1.5 g/d of the drug for > 30 d^[89]. Thus, monitoring the neurological state of the patient during metronidazole therapy is strongly encouraged^[88]. Both demyelinating and nondemyelinating neuropathies can be observed^[89]. Metronidazole-induced neuropathies are characterized by sensory manifestations with occasional ataxic features and generally are transient and resolve completely on discontinuation of medication^[14,89]. Peripheral neuropathies are one of the most frequent neurological complications described in IBD patients^[78]. The incidence of spontaneous peripheral neuropathy in IBD patients varies from 0.9%^[2] to 3.6%^[11]. These conditions are also described in other sections of this paper as side effects of biological therapies and in autoimmune nervous system disorders. Indeed, polyneuropathies in IBD may result from multiple interactions between immune-mediated disorders, nutritional imbalances, malabsorption, weight loss, vitamin deficiencies and drug-induced changes^[89]. The most important symptoms of peripheral neuropathy are paresthesia and increased threshold for temperature detection (the last sign is indicative of early neuropathy)^[88]. Axonal polyneuropathy usually is characterized by sensory loss and dysesthesia in a glove-and-stocking distribution, and decreased or absent ankle jerks with infrequent motor involvement^[84]. Small fiber nondemyelinating sensory neuropathy is characterized by subjective numbness and tingling in the absence of demonstrable abnormalities on electromyography and nerve conduction studies^[72], and may be misdiagnosed as fibromyalgia upon typically normal electrophysiological testing^[14]. IBD patients with restricted sensory involvement are usually younger than those with concomitant involvement of motor and sensory large fibers^[90]. Only some of these patients (particularly patients with CD) have been previously treated with metronidazole, and the disease progressed also after drug discontinuation, suggesting its contributory but not causative role^[76]. The physician should suspect peripheral neuropathy in cases of sensitive disturbances of the upper and lower extremities, ataxia and/or impairment of walking^[84].

Chatzkel *et al*^[91] described a 15-year-old girl affected by CD with ataxia and dysmetria 7 d after initiation of

treatment with metronidazole. Cranial MRI revealed bilateral symmetric T2/FLAIR hyperintense lesions of the dentate nuclei without contrast enhancement or restricted diffusion, and the lesions disappeared completely after drug discontinuation^[91].

Cyclosporine A

Cyclosporine A is a cyclic polypeptide that interferes with the transcription of cytokines, causing the blocking of activation and maturation of various cell types involved in cell-mediated immunity^[92]. This drug has been mostly used in severe refractory UC. Neurotoxicity is one of the major adverse events of this treatment, involving up to 25% of treated patients and including seizures, tremors, paresthesia, ataxia, motor deficits, aphasia, altered consciousness, and various degrees of visual and oculomotor disturbances^[92]. The pathogenesis of these neurological side effects is poorly understood. Cyclosporine A may rarely cause accelerated hypertension leading to progressive reversible encephalopathy syndrome^[14]; this condition is more frequent in patients with low total serum cholesterol^[14].

Irreversible bilateral optic neuropathy has also been described^[92] and two possible mechanisms have been proposed: direct toxicity to peripheral nerves, or thromboembolism leading to ischemic optic neuropathy^[93].

Cerebellar atrophy^[93] caused by cyclosporine A therapy performed despite hypomagnesemia may have its first manifestation in nystagmus. However, cyclosporine-A-induced neurotoxicity has also been described in the absence of known risk factors such as hypocholesterolemia, hypomagnesemia, previous seizure disorders and arterial hypertension^[94]. The differences in neurological side effects between oral and intravenous cyclosporine A is another matter of uncertainty^[95]. Cyclosporine A is insoluble in water and intravenous formulations are prepared in a polyoxyethylated (POE) castor oil and ethyl alcohol solution^[96]. In *in vitro* experiments, 0.1% POE castor oil determines axonal swelling and degeneration while 0.001% POE castor oil may induce demyelination. It has thus been suggested that residues of ethylene or its polymerization products might at least contribute to the neurotoxicity of intravenous cyclosporine A *in vivo*. However, the Cosmetic Ingredient Review Expert Panel has concluded that these cosmetic ingredients (POE castor oil and its derivatives) are safe in practical use^[96] and no serious neurotoxicity may be attributable to them.

Azathioprine

This drug has no specific neurotoxicity but it represents a predisposing factor to infection, particularly associated with its prolonged use. Spinal epidural abscess has been described as a complication of azathioprine therapy^[97]. Spinal epidural abscess is a rare but known neurological complication of CD^[97]; predisposing conditions are immunosuppressive therapy and the presence of intra-abdominal and/or retroperitoneal fistulas^[97]. A high index of suspicion should be raised if the patient shows back pain during or immediately after a flare of CD, with or

without neurological signs, because this condition is an alarm sign for the presence of inflamed paravertebral and spinal structures^[89]. Spinal epidural abscess represents a neurosurgical emergency in the presence of unresponsive back pain or progressive neurological deterioration such as bowel and/or bladder dysfunction. In patients with spinal epidural abscess associated with bowel fistulas and psoas abscesses determined by CD, a combined and highly specialized medical and surgical approach is needed to prevent recurrence^[98]. Prolonged antibiotic use is recommended also if cultures are negative.

Murai *et al.*^[99] reported a myelo-radiculitis determined by *Cryp. neoformans* in a UC patient on immunosuppressive therapy with azathioprine. Robineau *et al.*^[100] reported a case of HSE related to azathioprine therapy in a 28-year-old woman treated for 4 years^[100]. The diagnosis was based on the presence of photophobia, headache, asthenia and nausea associated with nuchal rigidity; lumbar puncture revealed a marked increase of lymphocytes (98%) in cerebrospinal fluid with a detection of HSV-1 DNA determined by PCR. Intravenous acyclovir administration (15 mg/kg every 8 h) for 3 wk completely resolved this complication.

CEREBROVASCULAR DISEASES

In general, the risk of both arterial and venous thrombosis^[101], as well as of thromboembolic events, is significantly increased in IBD patients. As a consequence, thromboembolic complications have been reported in various organs, including the brain. Indeed, cerebrovascular disorders have been documented in 0.12%-4% of all IBD patients, and probably they represent the most frequently reported neurological complications^[102]. Obviously, cerebrovascular or cardiovascular events and their sequelae may be of particular severity, especially if one considers the usually young age of IBD patients. The relative risk of stroke is higher in young patients, especially women and patients with CD^[14]. Thus, a large number of studies addressing the possible underlying causes of this predisposition in IBD and proposing possible prophylactic and therapeutic strategies have been published in recent decades. As a whole, these studies suggest that active disease, even at an outpatient level, appears to be the most important predisposing factor, through many pathogenic factors activated by the ongoing inflammation. Indeed, the intimate inter-relationship between inflammation and coagulation has become clear in recent years with disease clinical and subclinical activity^[103] shown to be associated with hypercoagulability related to various factors such as qualitative and quantitative abnormalities of platelets^[104] and coagulation factors^[94], decreased anticoagulant activity^[100], hypofibrinolysis^[105], malabsorption and hypercatabolism leading to vitamin B6 deficiency^[106], endothelial changes^[102] leading also to reduced activation of protein C, dehydration, and corticosteroid therapy^[107].

The abundance of the findings showing an association between clinical activity and risk of thrombotic events has led to the introduction of antithrombotic

prophylaxis in the therapeutic guidelines of hospitalized patients with severe relapse.

However, although active disease is particularly associated with an increased risk of these complications, some cases of vascular accidents have been described during remission^[101], suggesting that IBD represents a risk factor for thrombosis. The search for a possible genetic association between IBD and carriage of factor V Leiden, G20210A prothrombin and methylene tetrahydrofolate reductase mutations has provided negative results^[102], thus suggesting that other, probably acquired although not related to inflammation, factors may indeed play a more important role. One of these factors might be hyperhomocysteinemia^[80,81], which may derive from a lack of attention to nutritional status, although in many cases the presence of subclinical inflammation cannot be ruled out. It is important to consider a higher risk in postoperative state associated with the development of arterio-arterial embolism, cardioembolism, and *in situ* cerebral thrombosis^[14].

Arterial thromboembolism

An increased risk of these complications is observed in patients with active UC and particularly in those with total colitis^[108], even if active disease is associated with an increased risk, some cases have been described during remission^[100]. Men and women are equally affected. The cerebrovascular involvement appears to be more frequent among younger IBD patients, as reported by Houissa *et al.*^[101] who described four cases of arterial thrombosis in IBD and three of these were younger than 25 years. Intestinal inflammation may lead to increased risk for thrombosis through several pathways: by activating the coagulation cascade; decreasing anticoagulant activity; and inducing hypofibrinolysis, malabsorption and hypercatabolism with vitamin deficiencies that may lead to hyperhomocysteinemia - a well known risk factor for thrombosis^[101]. Also dehydration, immobility, sepsis, surgery and corticosteroid therapy may determine cerebral thrombosis in IBD patients^[101].

The neurological presentation of cerebral arterial thrombosis may vary from headache (95%), to mono- or bilateral paresis (43%), general or focal seizures (47%), or dysphasia (37%)^[104]. The clinical sequelae of cerebral vascular thrombosis can be devastating, especially in young patients with active and complicated IBD, leading to high mortality and disability in about 60% of cases^[101]. Conventional CT or MRI identifies the exact site of cerebral affected areas. At present, no guidelines are available for the treatment of cerebral thrombosis and stroke in IBD^[108]. Low molecular weight heparin (LMWH) is the most common drug used for the prophylaxis and treatment of vascular thromboembolism.

Given the heightened risk of thromboembolism in patients with IBD, prophylaxis with LMWH is recommended in hospitalized IBD patients, considering exacerbation of the disease^[14]. Long-term use of anti-

coagulant therapy in the treatment of arterial ischemic cerebral lesions is limited, although the presence of a hypercoagulable condition should always be considered an indication for lifelong anticoagulation with warfarin^[107]. Thrombolysis with recombinant tissue plasminogen activator, urokinase or streptokinase should also be considered in early cerebral arterial ischemic conditions (within 3 h from development of clinical symptoms) and this procedure, in expert hands, may be considered safe and effective^[109]. In selected cases, thrombectomy should also be considered^[110]. Rapid evaluation and appropriate multidisciplinary consultation are required for optimal diagnosis and management.

Venous and sinus thrombosis

Cerebral venous and sinus thrombosis is a rare condition and accounts for about 1% of all strokes^[111]. Cerebral venous and sinus thrombosis that concurrently develops with UC is rare^[111] and they may be associated with abnormalities in the coagulation system^[100]. Cerebral venous thrombosis appears to be more common in UC than in CD patients^[112], and is more commonly localized in the superior sagittal sinus and lateral sinuses^[113], although cortical venous thromboses have also been reported^[113].

All patients with UC and cerebral venous thrombosis reported in literature are young, mostly men, without other risk factors^[102]. Most patients have a pancolitis suggesting a role for increased endotoxemia and dehydration^[102] as culprits for vascular thrombosis. The most frequent symptom is headache occurring in 75%-96% of patients^[114]. The headache is often severe and diffuse and it usually precedes the appearance of neurological signs. A combination of focal defects, headache, seizures and altered consciousness is suggestive of cerebral venous thrombosis^[114], although the presenting features are variable and the condition should be considered in any IBD patients with neurological symptoms, particularly during an active phase. Cerebral infarction is a dangerous complication and it appears when the thrombosis extends from the superior sagittal sinus to the superficial cerebral veins and their tributaries^[114].

MRI studies in combination with MR venography are sensitive in identifying venous sinus occlusion^[114]. The use of local endovascular thrombolytic agents may restore the flow more frequently and rapidly than heparin alone; however, there is no evidence of the superiority of this method and the risk of hemorrhage is high^[114]. Warfarin is usually continued for at least 6 mo after a first episode of cerebral venous thrombosis, or longer in the presence of persisting predisposing factors^[114]. The theoretical risk of intestinal bleeding due to anticoagulant therapy does not appear significant in practice^[114]. Given the increased risk of thromboembolism in patients with IBD, aggressive mechanical and pharmacological deep vein thrombosis prophylaxis with LMWH is recommended in hospitalized patients^[14].

IMMUNE-MEDIATED NEUROLOGICAL DISORDERS

MS

This topic has been already treated in this paper as a possible complication of biological therapies^[25]. However, a possible spontaneous association between MS and IBD has been suspected for decades^[115]. Indeed, the estimated prevalence of MS in the general population is about 0.1%, while in IBD patients the prevalence of MS has been reported at up to 0.5%^[8,114,115], suggesting a 1.5-5-fold increase in the risk of having MS in IBD patients^[30]. In evaluating these data, however, we should bear in mind that all studies involved a limited number of patients, thus yielding low statistical power. Also, results of the studies are greatly influenced by the methods used to look for an associated disease. Indeed, Geissler *et al.*^[116] observed hyperintensity of the white matter on brain MRI in almost half of the patients with IBD free of neurological symptoms compared to only 16% of healthy age-matched controls. MS has been reported to develop either before or after the clinical onset of IBD^[116]. The nature of a possible pathogenic link between IBD and MS has not been identified, but a disturbance in functional T-cell subsets with aberrant proinflammatory activity of T helper 17 subsets has been suggested^[2]. Animal studies^[117] also suggest a link between the demyelinating lesions suggestive for MS or acute disseminated encephalomyelitis and the prothrombotic state characteristic of UC. Astrocytosis and extensive perivenular loss of myelin have been described in rhesus monkeys suffering from colitis and cerebral venous thrombosis, and it is possible to speculate that the demyelinating lesions could have been the result of perivenular edema secondary to venous blockade^[117]. Indeed, cerebral lesions in the monkey are identical to those observed in confluent leukoencephalitis and perivascular myelosis of the cerebral type; a demyelinating disease of monkeys^[118]. Whatever the mechanisms of the underlying possible association between MS and IBD, brain MRI followed by a neurological consultation for further diagnostic work-up should be organized^[2] as soon as a patient with a clinical history of UC or CD presents with an unexplained neurological symptom suggestive of MS, such as paresthesia in both arms, fingers, legs and toes, hyperesthesia of the fingertips and hyper-reflexia. Occasional, but clinically important, observations are those reporting that a demyelinating disease may be precipitated or aggravated by the use of infliximab for IBD^[119].

Cerebral vasculitis

This topic has already been treated in the chapter on complications of biological therapies^[22]. Cerebral vasculitis has been reported in association with UC^[120] and can be considered a further cause of stroke. The association between UC and Takayasu's disease, particularly in Japanese patients with an HLA-B52, DR2 haplotype, is strong^[121]. The pathogenetic process may be related

to common immune-mediated mechanisms such as T-lymphocyte mediated cytotoxicity or immune complex deposition^[121], or to a genetic susceptibility determined by patient's HLA status. The association of UC with perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) or atypical ANCA^[122] suggests a common autoimmune etiology, but the existence of different antigenic recognitions by these antibodies in the two diseases is well established. Furthermore, UC-associated ANCA lack antigenic specificity for proteinase-3 (PR3) or myeloperoxidase (MPO) and do not have the potential for the development of systemic vasculitis or for neutrophil activation^[122]. Nevertheless, a p-ANCA (specific for MPO) positive UC patient affected by ischemic lesions in the white matter of the brain has been reported^[122].

The clinical manifestations of cerebral vasculitis are hemiparesis, hemianopsia, personality changes, headache, aphasia, seizures, coma and progressive dementia^[8]. These clinical manifestations may occur independently of the activity of the underlying bowel disease and, in some cases, they may appear even before the onset of IBD^[123]. Cerebral MRI is always abnormal^[8]. Nearly half of the reported patients have neurologic signs and symptoms developing during steroid therapy^[8].

Necrotizing angitis may show clinical manifestations similar to the acute hemorrhagic variant of acute disseminated encephalomyelitis (ADEM)^[117]. ADEM is suggested to result from a transient autoimmune response directed against myelin or other autoantigens via molecular mimicry^[117] caused by a defective epithelial barrier function in UC, leading to uncontrolled uptake of luminal antigens and stimulation of pathologic immune and inflammatory reactions^[8]. The presence of lethargy should always suggest a diagnosis of ADEM.

Autoimmune myelopathy

Lossos *et al.*^[3] reported nine UC patients with neurological disorders and six of these had peripheral nerve disorders considered as acute inflammatory demyelinating polyradiculoneuropathy^[3]. This study was limited by the absence of detailed information about CSF features and response to therapy.

Myelopathy, which may present as a slowly progressive systemic spastic paraparesis in the absence of a spinal sensory level, has been associated with UC in some case reports^[3], a link with human T-lymphotropic type 1-associated myelopathy has been proposed^[124]. This syndrome may develop without spinal MRI abnormalities^[3], an immune-mediated inflammatory origin has been suggested but a possible association with the use of medications or nutritional deficiencies cannot be ruled out^[3]. It is possible to consider this myelopathy or transverse myelitis as a part of a more widespread CNS disorder like MS or a vasculitis process as suggested by Ray *et al.*^[125].

Also, *Campylobacter jejuni* is linked to exacerbations of IBD and it may contribute to the development of autoimmune inflammatory demyelinating polyneuropathy^[121].

It is noteworthy that in the patients with generalized peripheral neuropathy, a demyelinating pattern is present in 30%^[125].

Myasthenia gravis

Myasthenia gravis (MG) is a typical immune-mediated disease in which T-lymphocyte function is abnormal, the thymus is enlarged, and circulating acetylcholine receptor antibodies are found^[126]. MG is often associated with other autoimmune disorders such as alopecia, lichen planus, vitiligo and SLE^[127]; diseases that are also observed in association with IBD. Tsuchiya *et al.*^[127] described an association between thymic abnormalities and IBD, with the presence of acetylcholine receptor antibodies^[128]. Also, the lack of age-related involution of the thymus observed in MG has also been reported in UC^[127]. T cells obtained from the thymus of patients with MG and UC have reduced ratios of suppressor (CD8⁺) to helper (CD4⁺) T cells compared with control subjects^[128].

Diplopia and ptosis of the upper eyelid in IBD patients may be an initial manifestation of MG^[129]. For the possible pathogenic association between the two diseases, intriguing observations on their therapeutic management have been reported: Finnie *et al.*^[126] reported a case of a patient with both MG and CD complicated by perianal disease whose bowel disease improved after thymectomy for severe uncontrolled MG. In contrast, Gower-Rousseau *et al.*^[130] described a patient with MG and UC in whom MG symptoms improved after proctocolectomy. Foroozan *et al.*^[129] reported a 21-year-old man with UC and binocular diplopia and ptosis due to MG; both ocular and gastrointestinal symptoms improved after plasmapheresis, azathioprine, prednisone and mestinon^[3].

Autoimmune sensorineural hearing loss

Sensorineural hearing loss is probably an immunological manifestation of IBD^[131]. The clinical manifestations of the disease are often bilateral and progressive^[132]. The hearing level is unstable with periods of deterioration alternating to partial or complete clinical remission^[132]. In general, the tendency is for gradual evolution towards permanent hearing loss^[132]. Vestibular dysfunction symptoms such as disequilibrium and postural instability may accompany auditory symptoms and these symptoms may have a sudden onset^[133].

Hearing loss generally occurs between 2 mo and 17 years after the diagnosis of UC. Hearing loss may appear during both active and remission stages of the disease and it does not have a parallel evolution. Hearing loss, if not treated, is recurrent until leading to complete deafness^[133]. A more strict collaboration with ear, nose and throat specialists should be encouraged to research this condition of autoimmune inner ear. Kumar *et al.*^[134] noted, in a controlled audiometry study, a significant sensorineural hearing loss in UC patients compared with controls. A subclinical sensorineural hearing loss may also be present in CD patients^[135].

The clinical response to steroid and immunosup-

pressive therapy suggests that an autoimmune process causes inner ear impairment^[136]. This condition is most frequently bilateral but may also be unilateral. Aggressive treatment should be started as early as possible. At present^[134], it is impossible to have detailed information about the beginning and the time-course of hearing loss because this condition is underdiagnosed. Karmody *et al.*^[133] reported that patients with hearing loss performed their first medical evaluation usually 3 years after the first clinical manifestation.

MISCELLANEOUS

Peripheral neuropathies

Polyneuropathies have previously been dealt as frequent side effects of metronidazole therapy but they may also occur as spontaneous extraintestinal manifestations of IBD. Gondim *et al.*^[89] identified 33 patients (18 with CD and 15 with UC) affected by polyneuropathies. Male sex was highly predominant (78% in CD and 75% in UC). Neurological symptoms appeared long after the diagnosis of IBD. In 33% of CD patients and 40% of UC patients, the polyneuropathy was correlated with disease activity.

In CD patients, demyelinating neuropathy was present in five patients while a nondemyelinating neuropathy was present in the other 13: small-fiber polyneuropathy (SF-PN) in two and large-fiber axonal neuropathy (LF-PN) in 11. Four patients with UC showed peripheral demyelinating neuropathies. Eleven UC patients showed nondemyelinating neuropathies: four with SF-PN and seven with LF-PN. The diagnosis of SF-PN was obtained by means of skin biopsy^[89].

Oliveira *et al.*^[4] studied 82 IBD patients (31 with CD and 51 with UC). Five CD patients (4 women) (16.1%) had SF-PN and the first symptom was sensory abnormality. Weakness was mild and mostly located in the distal legs. Neurological examination showed decreased or absent ankle jerks, and decreased distal vibration and pinprick. Ten UC patients (19.6%) had mild axonal sensory motor polyneuropathies (SM-PN). Blood B12 levels were < 200 pmol/L in two of these 10 patients, between 200-300 pmol/L in a further two, two patients had diabetes, one was affected by hypothyroidism, and positive blood rheumatoid factor was present in two^[4]. Fourteen percent of UC patients were taking steroid therapy^[4].

Oliveira *et al.*^[4] concluded that SM-PN in UC patients was more common in women, in older individuals, in patients developing the disease later in life and in subjects with a body mass index < 18.5. The authors^[4] also noted that the association with autoimmune diseases such as diabetes mellitus, hypothyroidism and positive rheumatoid factor was more frequent in UC patients with SF-PN than in CD patients.

Sassi *et al.*^[137], in a study of 102 consecutive patients with IBD, reported nine patients (8.8%) with peripheral neuropathies. Bernstein *et al.*^[138], in a large study of administrative healthcare data from the Manitoba County

between 1984 and 2003 on 8072 patients with IBD (3879 with UC and 4193 with CD), reported peripheral neuropathies in 2.4% of UC patients and 2.34 of CD patients compared to 1.35% in the general population. Peripheral neuropathies usually do not respond to treatment of the underlying IBD^[14].

Cranial nerve palsies

Cranial nerve palsies can be observed in patients with IBD. The Melkersson-Rosenthal syndrome^[139] is defined by recurrent facial nerve palsy, fissuring of the tongue, and noncaseating tissue granulomas, and it has been described in association with CD^[140]. The long intracranial course of the sixth nerve predisposes it to injury by a variety of abnormalities^[139]. Karajeh *et al.*^[140] described a 27-year-old female smoker with a 12-d history of diplopia on right lateral gaze associated with retro-orbital pain before the clinical diagnosis of CD. This typical clinical presentation suggests a vascular sixth nerve palsy with a sudden onset of unilateral abduction deficit accompanied by retro-orbital pain and diplopia^[140]. It is hypothesized that microvascular ischemic demyelination of a portion of the nerve is the most likely cause of this clinical condition^[140]. This area of ischemic demyelination subsequently undergoes remyelination with clinical recovery^[140]. Complete recovery within 2-3 mo is generally observed^[140].

Optical neuropathy, as outlined above, has a clinical presentation with a bilateral optic disc swelling and it is a rare condition prevalently associated with CD^[141]. Romero Aroca *et al.*^[142] reported a 27-year-old woman affected by UC and optic neuritis that resolved after mesalamine administration. Optic neuropathy may be attributed to peripapillary inflammation, optic disc ischemia, or intracranial hypertension^[142].

A local vasculitis process or a general hypercoagulability condition can determine optic nerve ischemia. Modern imaging techniques usually allow one to exclude dural venous sinus thrombosis^[113]; a serious cerebrovascular complication of IBD described in another chapter of this paper. Another clinical condition is the severe erosive arthritis of the craniocervical junction that should always be considered in IBD patients with persistent neck pain because a late diagnosis may determine severe neurological defects^[143].

Epilepsy

The association between IBD and epilepsy is uncertain^[1]. Epileptic seizure in IBD patients may be related to structural or metabolic causes^[3]. Seizures may be generalized tonic-clonic complex, simple, partial or even multiple. A MEDLINE search^[8] using “epilepsy and ulcerative colitis” as keywords found only five case reports dating back to the early 1970s. According to the literature, epilepsy appears more frequently associated with CD than with UC^[1].

Muscle disorders

Granulomatous myositis and myopathies are associated with both CD and UC patients^[144]; these manifestations usually appear during exacerbations of IBD^[145]. Orbital

myositis is a nonspecific, localized orbital inflammatory process in which one or more extraocular muscles are involved^[145]. Clinically, orbital myositis is characterized by acute pain exacerbated by eye movements; diplopia, swelling of the eyelid, conjunctival injection, and exophthalmos may also be present^[145]. The diagnosis is based on clinical history and imaging^[145]. This disease responds to steroid therapy^[146]. Orbital myositis is rare in IBD^[146]; sarcoidosis, Wegener's granulomatosis, rheumatoid arthritis and Lyme disease should be considered in the differential diagnosis^[146].

Nonspecific orbital inflammation includes histological forms that are more difficult to distinguish such as an idiopathic granulomatous and idiopathic sclerosing pseudotumor^[147]. Nonspecific orbital inflammation appears to arise from an immune reaction in the orbit secondary to a neighboring zone of inflammation or a distant autoimmune reaction^[148]. It has been associated with CD, diabetes, rheumatoid arthritis and Graves' disease^[148].

MRI is the method of choice to study the orbital region in orbital myositis because it is able to show the typical diffuse enlargement of extraocular muscles with blurred margins and to rule out other lesions such as tumor/pseudotumor infiltration, apical extension, cavernous sinus involvement and intracranial disease^[146]. Orbital myositis most commonly affects the superior recti, the medial recti and oblique muscles.

Classic migraine

The prevalence of migraine in patients with IBD remains unknown^[149]. Migraine is associated with systemic endothelial dysfunction^[150], which is also proposed as a possible pathogenic factor in IBD^[151]. Oliveira *et al.*^[4] found that headache is the most common neurological complaint reported both in CD and UC patients, in 54.8% and 56.9% of patients, respectively. In most patients headache is not disabling and it is often associated with IBD relapse and treatment^[4]. Ford *et al.*^[149], in a study performed on about 100 IBD patients (77% women and 23% men, 66% with CD and 27% with UC) by an ID-Migraine questionnaire^[148], noted a 30% prevalence of migraine in IBD patients. Migraine was more prevalent in CD (36%) than in UC (14.8%). In UC patients, the prevalence of migraine in women did not approach that of the general population (12.5% *vs* 18.2%), whereas the prevalence in men greatly exceeded that of the general population (18% *vs* 6.5%)^[152].

Currently, migraine is underdiagnosed in IBD patients, although it causes limited ability to work, study and perform routine activities in a high percentage of IBD patients^[146]. It is important to consider that migraine with aura may be an independent risk factor for ischemic stroke in women^[153] because they have a 13.7-fold increased risk for silent infarction in the posterior territory and 2.1-fold increased risk for deep white matter lesions^[154].

Sleep disturbances, depression and anxiety, chronic fatigue syndrome

Sleep disturbances are recognized reactions to inflamma-

tion^[155] and may represent the first response to acute inflammation^[156], although they may persist during clinical remission.

Depression and anxiety occur in IBD and they may involve sleep disturbances and asthenia^[156]. Assessment of depression and anxiety in IBD is mandatory because these conditions may contribute to the subjective perception of poor quality of life^[157]. Anxiety may be associated with more intense disease activity^[158].

Fatigue in IBD may be considered as a consequence of the disease and its treatment^[157]. Iron deficiency may cause fatigue and sleep disorders in patients with CD^[157]. Patients can express fatigue even when bowel disease is inactive^[159]. Minderhoud *et al*^[160] showed that the fatigue score remains high during disease remission compared with normal control subjects. Lipton *et al*^[152], in a study of French IBD patients, found that the scores obtained on the Multidimensional Fatigue Inventory in IBD patients were similar to those affected by cancer. Fatigue may be considered a part of the core symptoms of depression^[161] and the use of antidepressants may improve chronic fatigue syndrome. In summary, although chronic fatigue syndrome is frequent in IBD patients, its precise pathogenesis is not clear and this most probably reflects a multifactorial nature of the syndrome.

Restless legs syndrome

Restless legs syndrome (RLS) is a CNS disorder characterized by a compelling urge to move the legs at rest; it contributes to sleep disturbances and impaired quality of life. RLS may be primary (idiopathic and familial) or secondary to many disorders such as pregnancy, end-stage renal failure, iron deficiency anemia, rheumatoid arthritis, diabetes, Parkinson's disease, fibromyalgia, IBD^[162], gastric resection, chronic liver disease, and irritable bowel syndrome^[163]. The diagnosis of RLS must be made according to the following four criteria established by the International RLS Study Group^[164]: (1) an urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs; (2) the urge to move or unpleasant sensations begin or worsen during periods of rest or inactivity; (3) the urge to move or unpleasant sensations are partially relieved by movement at least as long as the activity continues; and (4) the urge to move or unpleasant sensations are worse in the evening or night than during the day or only occur in the evening or night.

Weinstock *et al*^[162] reported a prevalence of 30% for RLS in patients with CD and noted that it appeared during or after the onset of CD symptoms, suggesting a link between CD and RLS. This association might at least partly explain the presence of both fatigue and sleep disturbances in CD patients^[161]. Patients with iron deficiency anemia are at particularly high risk of developing RLS^[165] because low brain iron concentration may play a role in altered dopamine levels, providing a unifying condition for most cases of the syndrome^[166]. In fact, inflammatory conditions such as CD cause an increased secretion of proinflammatory cytokines [*i.e.*, interleukin

(IL)-6]^[167] which in turn causes increased hepcidin production^[163], leading to iron deficiency in the CNS as a cause of RLS^[168]. Small intestinal bacterial overgrowth, which may be observed in CD patients may also cause RLS by an inflammatory state supported by IL-6 and hepcidin as in MS patients^[169]; in these cases, antibiotic therapy might be beneficial^[169].

Wernicke encephalopathy

Wernicke encephalopathy is a neurological complication determined by vitamin B1 deficiency^[170]. Hahn *et al*^[170] have reported vitamin B1 deficiency in a young patient with CD receiving total parenteral nutrition without vitamin replacement. Larnaout *et al*^[171] reported a CD patient with Wernicke encephalopathy under normal enteral nutrition. This patient died and autopsy revealed the following pathological cerebral lesions: hemorrhagic necrosis around the third and fourth ventricles with vascular proliferation and pericapillary hemorrhages; numerous small hemorrhagic infarctions in the central part of the corpus callosum; marked spongiosis, predominantly in the left cerebellar white matter; slight thickening of leptomeninges with some mononuclear cells; and absence of vascular thrombosis or inflammatory perivascular cuffing in the brain and spinal cord.

Vitamin B12 deficiency

Vitamin B12 deficiency due to terminal ileal disease or surgical resection in CD may cause subacute myelopathy combined with degeneration characterized by bilateral spastic paresis, loss of pressure and vibration sensation due to degeneration of the posterior and lateral columns of the spinal cord^[172].

CONCLUSION

Neurological complications of IBD, either related to drug therapy or spontaneously associated with the disease, are relatively frequent and may contribute to a high degree of morbidity and permanent damage. They are also frequently difficult to recognize and diagnose, due to their frequently unclear clinical expression. For these reasons, knowledge of the different presentations as well as of differential diagnosis and therapeutic possibilities is important for the gastroenterologist dealing with IBD patients. This paper is thus aimed at providing interested physicians with an in-depth review of the main features of neurological complications of IBD.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Primary sclerosing cholangitis as an independent risk factor for colorectal cancer in the context of inflammatory bowel disease: A review of the literature

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Abstract

To examine and evaluate recent evidence regarding the epidemiology, pathogenesis and management of colorectal cancer (CRC) development in inflammatory bowel disease (IBD)-primary sclerosing cholangitis (PSC) patients. Using the PubMed database, a literature search was conducted for relevant articles in English from the past 10 years. Relevant studies investigating PSC as a risk factor for CRC in IBD in the context of incidence and prevalence, pathogenesis, prevention and prognosis were included in this review. Recent evidence increasingly points to PSC as a significant risk factor in the development of CRC in patients with concomitant IBD. PSC may be an important risk factor for CRC in different populations worldwide. The mechanism for this increase in risk is still unclear. The efficacy of UDCA as a chemopreventive agent remains controversial. Liver transplantation does not halt the development of CRC, although there is not enough evidence to suggest that it is associated with increased

incidence of CRC. While routine colonoscopic surveillance should be performed in patients with concurrent PSC and IBD, more high-level evidence is required to support the benefits of the procedure. While many new developments have taken place in the last decade, the pathogenesis and optimal management of CRC development in IBD-PSC patients remain unclear.

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Key words: Primary sclerosing cholangitis; Ulcerative colitis; Crohn's; Inflammatory bowel disease; Colorectal cancer; Liver transplantation; Ursodeoxycholic acid

Core tip: The widely accepted risk factors for malignant transformation in inflammatory bowel disease (IBD) are disease duration and extent of inflammation. Since first proposed in 1992, one increasingly recognised independent risk factor for colorectal cancer development in IBD patients is concomitant primary sclerosing cholangitis.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a widely accepted risk factor for colorectal cancer (CRC). The development of CRC complicating IBD only occurs in 1%-2% of CRC cases and has been reported to account for up to a third of mortality in ulcerative colitis (UC) patients^[1].

Table 1 Summary of studies investigating colorectal cancer as a risk factor in patients with concurrent primary sclerosing cholangitis and inflammatory bowel disease

Ref.	Location	IBD-PSC patients (n)	Colorectal neoplasms (n)	Matched controls?	Study type	Is IBD-PSC a risk for CRC?
[24]	The Netherlands	IBD (126)	CRC (16)	No	Retrospective	Yes
[9]	Belgium	IBD (107)	CRC (10)	No	Retrospective	Yes
[12]	Sweden	IBD (152)	CRC/Dys (3)	No	Retrospective	No
[31]	Germany	IBD (120)	CRC (7)	No	Prospective	Only with dominant stenosis
[28]	Argentina	UC (39)	CRC (7)	Yes	Prospective	Yes
[30]	United States	UC (50)	N/S	Yes	Retrospective	No
[32]	Sweden	CD (28)	CRC/Dys (9)	Yes	Retrospective	Yes
[33]	United Kingdom	CD (35)	Dys (1)	No	Retrospective	No

UC: Ulcerative colitis; CD: Crohn's disease; Dys: Dysplasia; N/S: Not specified; CRC: Colorectal cancer; PSC: Primary sclerosing cholangitis; IBD: Inflammatory bowel disease.

Established risk factors for malignant transformation in IBD include disease duration and extent^[2-4], family history of CRC^[5,6], and concomitant primary sclerosing cholangitis (PSC).

PSC is a chronic syndrome of unknown aetiology. PSC is characterised by destruction and stenoses of intrahepatic and extrahepatic biliary ducts by inflammation and fibrosis, leading to cholestasis. As the disease progresses, portal tract fibrosis and biliary cirrhosis may develop, which may ultimately lead to death from hepatic cirrhosis and failure^[7]. Besides CRC, PSC has been associated with other malignant conditions, including cholangiocarcinoma^[8,9], pancreatic carcinoma^[10], gallbladder cancer^[11], hepatobiliary cancer^[12], and hepatocellular carcinoma^[10,13].

The incidence of PSC may be increasing, possibly due to earlier recognition and increasing index of suspicion^[14]. The mean age of PSC diagnosis is 40 years, and the median survival time from diagnosis to death or liver transplantation is approximately 12 years^[15]. PSC has a slightly male predominance^[16]. Currently, the only definitive long-term treatment of PSC is liver transplantation^[7].

The association of PSC with UC is stronger than with Crohn's disease (CD). The prevalence of concurrent PSC in UC patients is up to 8%, compared to only 1% to 3% for CD^[17,18]. Evidence suggests that this figure varies according to the extensiveness of disease; the prevalence of PSC is approximately 5.5% in patients with pancolitis, but only 1% in those with distal colitis^[18,19]. Overall the prevalence of PSC is approximately 10% in CD and 80% in UC^[19,20]. Broomé *et al.*^[21,22] first proposed the association of PSC and CRC in UC patients in 1992 with a cumulative risk of 50% at 25 years of developing CRC in UC = PSC patients.

The suggestion that PSC is an independent risk factor for CRC in IBD patients is one that has widely debated. Currently, no explanation of how PSC increases the risk of CRC in IBD patients has been agreed upon. Numerous literature reviews, including a meta-analysis conducted in 2002^[23], have evaluated earlier research concerning this topic. This review aims to evaluate research within the last decade and examine recent evidence concerning epidemiology, pathogenic mechanisms

and management strategies of CRC development in IBD-PSC patients.

RESEARCH

A literature search was conducted using the PubMed database for relevant articles from January 2002 until January 2014. The keywords used were: primary sclerosing cholangitis, colorectal, cancer, neoplasia, carcinoma, inflammatory bowel disease, Crohn's disease and ulcerative colitis. Relevant studies investigating PSC as a risk factor for CRC in IBD in the context of incidence and prevalence, pathogenesis, prevention and management, and prognosis were included in this review. Articles not written in English, review articles and published abstracts were not included.

EVALUATION OF EVIDENCE

Since the initial proposal in 1992 by Broomé *et al.*^[21], recent research continue to support concurrent PSC as a key risk factor in the development of CRC in IBD. The studies evaluated in this section are summarised in Table 1. Many studies now recognise PSC as just an important risk factor for CRC development as previously established risk factors such as duration and extent of IBD.

Early studies of PSC patients were characterised by small cohorts, small statistical power and disparate measurable outcomes. The increasing awareness of the link between PSC and CRC has allowed larger and better designed studies to be conducted. A retrospective Dutch study in 2009 investigated 211 PSC patients. Of that cohort, 60% had concurrent IBD. The risk of CRC development was 14% at 10 years and 31% at 20 years in PSC patients with concurrent IBD, compared with a steady risk of 2.3% in patients without concurrent IBD ($P < 0.01$)^[24]. The study also found that the majority of CRCs were located in the right colon, proximal to the splenic flexure, a result that has been confirmed by numerous other studies^[25,26]. The same group went on to confirm this finding in a subsequent study, where the majority (67%) of IBD-PSC patients that developed CRC had tumours in the right-sided colon ($P < 0.01$) in contrast to patients with IBD alone. Based on this finding, a differ-

Table 2 Summary of studies investigating the efficacy of ursodeoxycholic acid a chemopreventive agent in primary sclerosing cholangitis patients with concurrent inflammatory bowel disease

Ref.	Location	UDCA (n)	CRN incidence UDCA (n)	No UDCA (n)	CRN incidence no UDCA (n)	Study type	Is UDCA chemopreventive?
[36]	United States	29	3	23	8	RCT	Yes
[37]	United States	28	3	92	13	Retrospective	No
[38]	Sweden	37	13	40	15	RCT	No
[39]	United States	25	9	31	3	RCT	No-high dose UDCA
[40]	Germany	120	7	N/A	N/A	Prospective	No-short term; yes-long term

CRN: Colorectal neoplasm (dysplasia and cancer); RCT: Randomised controlled trial; N/A: Not applicable; UDCA: Ursodeoxycholic acid; PSC: Primary sclerosing cholangitis; IBD: Inflammatory bowel disease.

ence in pathogenesis of CRC may occur in patients with PSC and concurrent IBD, compared to patients with IBD alone^[27].

Other studies have also evaluated the cumulative risk of CRC development in the long-term. Fevery *et al*^[9] studied 200 PSC patients in a long-term, single-centre study in Belgium, where 60% of the cohort had concomitant IBD. The cumulative incidence for the diagnosis of CRC after IBD diagnosis was found to be 2% in 5 years, 7% in 10 years and 15% in 20 years. Additionally, the median age of CRC diagnosis was 49.5 years, leading the authors to conclude malignancy to be the major cause of early mortality in patients with PSC.

Terg *et al*^[28] prospectively recruited a Latin American PSC cohort from 1333 patients with UC. The prevalence of PSC was 2.9% and the cumulative risk of CRC after 10 and 20 years in these patients was 11% and 18% respectively, compared to 2% and 7% in UC without PSC ($P < 0.01$). Hence, it was confirmed that patients with UC and PSC indeed have a higher risk of CRC. An Asia-Pacific Consensus Group consisting of representatives from a host of countries, including India, China, Philippines and Australia published a paper in 2010 outlining various findings in UC patients in these countries. There was consensus on the statement that PSC associated with UC is less prevalent in the Asia-Pacific region compared to Western nations, though the level of evidence on this finding was classified as fairly weak. There was also some consensus on the statement that PSC in the setting of UC significantly increased the risk of development of CRC^[29].

Some studies have not confirmed PSC as an independent risk factor of CRC in IBD. A 2006 case-control study investigating predictive and protective risk factors associated with CRC in UC patients did not find PSC to be significant^[30]. A Swedish population-based cohort of 199 PSC patients revealed that while the disease was associated with a four-fold increase in mortality compared with the general population (SMR = 4.20, 95%CI: 3.01-5.69), the researchers unexpectedly could not confirm that PSC or PSC with concurrent IBD was associated with a higher incidence of CRC and colorectal dysplasia compared with the general population^[12]. This cohort of patients was diagnosed from 1992 to 2005, which is relatively recent compared with other large-scale studies of PSC patients with cases recruited from

the 1980s^[24]. This led the researchers to postulate that the lowered incidence of CRC in this cohort was a result of better management of IBD in recent years^[12].

Studies have also disease subtypes in the prediction of CRC. A prospective study of 171 PSC patients being treated with ursodeoxycholic acid (UDCA) found that IBD coexisting with dominant ISC bile duct stenosis had an increased CRC incidence, whereas IBD without dominant stenosis had no effect on the incidence of carcinoma ($P < 0.05$). The authors did not speculate whether this observation was a result of the interaction between dominant stenosis and IBD, or whether it was to do with UDCA treatment^[31].

While the risk of CRC is established for UC-PSC patients, studies have also evaluated their association with CD. The overall risk of CRC development in CD-PSC is not as strong as UC-PSC. Lindström *et al*^[32] studied the development of CRC in 28 patients with both PSC and CD, compared to controls with CD only. They found PSC to be a risk factor for development of CRC and dysplasia in CD (OR = 6.78, 95%CI: 1.65-27.9), but the study was limited by its small cohort and retrospective design. Another retrospective review of 166 PSC-IBD patients did not find an increased risk of CRC or dysplasia in CD^[33].

PREVENTION OF CRC

The cause of the increased risk of CRC in PSC is largely unknown. Studies have evaluated whether the risk of CRC can be reduced. Strategies such as colonoscopic surveillance, UDCA and liver transplantation have been investigated as potential methods of preventing the development of CRC.

UDCA

The chemopreventative effects of UDCA against CRC in IBD-PSC patients remain controversial. UDCA is a synthetic, hydrophilic bile acid that purportedly prevents the carcinogenic effects of secondary bile acids in the colon^[34,35]. A summary of recent data concerning the efficacy of UDCA is presented in Table 2.

A randomised, placebo-controlled trial evaluated the effect of UDCA on CRC and colorectal dysplasia in patients with concurrent UC and PSC^[36]. Colorectal neoplasia developed in 10% of the patients assigned to the

UDCA group compared to 35% of the patients assigned to the placebo group (RR = 0.26, 95%CI: 0.06-0.92). Wolf *et al.*^[37], however, reported that the incidence of CRC and colorectal dysplasia was not significantly different between patients treated with UDCA and patients that were not, but the UDCA patients did report a lower mortality rate ($P < 0.05$).

A long-term, randomised placebo-controlled trial of IBD-PSC patients prescribed UDCA *vs* placebo followed-up patients for more than 10 years yielded no difference in the CRC rate between the UDCA (13%) and placebo (16%) groups. There also was no significant difference in cancer-free survival between the two groups^[38]. Another long-term, randomised placebo-controlled trial assessed the effects of high dose UDCA (28 to 30 mg/kg per day) on the development of colorectal dysplasia and CRC in UC-PSC patients. The study found that UDCA had an adverse effect on neoplasia development where high dose UDCA significantly increased development of colorectal dysplasia and CRC compared to control (HR = 4.44, 95%CI: 1.30-20.1)^[39].

Some studies have attempted to reconcile these conflicting findings. In a prospective cohort study conducted by Rudolph *et al.*^[40], the trend of colorectal carcinoma development in patients treated with UDCA was observed to increase up to 6 years after the start of treatment, plateaued between 6 to 9 years, and after treatment for more than 9 years (up to at least 12 years) no further colorectal carcinomas developed. This finding, together with others, suggests that the effects of UDCA in UC-PSC patients may not be straightforwardly beneficial or non-beneficial, and that longer term, placebo-controlled trials are needed to provide evidence for or against UDCA as a chemopreventative agent. Lower doses of UDCA have been used to avoid possible adverse events.

A recent meta-analysis reporting 177 cases of CRC in 763 patients with PSC-IBD failed to demonstrate significant protective association between UDCA use and CRC with OR = 0.81, 95%CI: 0.41-1.61. However, a significant chemopreventive effect was found on the risk of advanced neoplasia defined as CRC and/ or high-grade dysplasia (OR = 0.35, 95%CI: 0.17-0.73). Low-dose UDCA (8-15 mg/kg per day) did significantly reduce CRC (OR = 0.19, 95%CI: 0.08-0.49)^[41]. Another meta-analysis of a similar dataset showed similar results^[42].

Liver transplantation

Currently, liver transplantation remains the only effective treatment of PSC with end-stage liver disease. It follows that liver transplantation in PSC may offer prevention against CRC development by improving PSC status, however, the evidence is largely contrary.

A study from the United Kingdom identified 152 patients with PSC following liver transplantation. Of these patients, 5.3% developed CRC, of which all of them had concurrent IBD with an intact colon. The cumulative risk of CRC development in IBD-PSC patients was calculated to be 14% at 5 years and 17% at

10 years. The risk of developing CRC in PSC patients without IBD was 0% at 10 years^[43]. Another study from the Cleveland Clinic found that coexistent IBD and PSC had a colorectal neoplasia incidence rate of 34% following liver transplantation, very similar to the incidence in matched IBD-PSC controls without liver transplantation (30%). However, the rate of colorectal neoplasia in IBD-PSC patients following liver transplantation was higher than if liver transplantation was performed for non-PSC indications (34% and 0%, respectively; $P < 0.05$)^[44].

In a large-scale study, Dvorchik *et al.*^[45] identified 192 patients with both PSC and IBD, and found no increase in the risk of CRC in these patients ($P < 0.001$). van de Vrie *et al.*^[46] conducted a retrospective study of patients having had liver transplantation for PSC, and concluded that transplantation was not adversely affected by IBD, nor was the course of IBD different after liver transplantation. High incidence rates of CRC remain following liver transplantation for PSC according to a recently published meta-analysis. The incidence rates of CRC were 5.8 per 1000 person-years but increased to 13.5 per 1000 person-years in those with an intact colon at the time of transplantation. A long duration of IBD and extensive colitis were confirmed as risk factors for CRC but specific transplant-related factors that may increase CRC risk were not identified^[47]. Overall, the evidence suggests that liver transplantation does not offer protection against CRC in PSC patients with concomitant IBD, and that post-liver transplantation patients are just as likely to develop CRC as non-transplanted patients. However, there is a lack of evidence to suggest that liver transplantation is an added risk factor for CRC development in IBD-PSC patients.

Surveillance

With increasing evidence conferring the increased risk of CRC in IBD-PSC patients, the importance of colonoscopic surveillance after the diagnosis of PSC in IBD patients has been stressed. The general consensus is that routine surveillance colonoscopy and random biopsies should be performed one to two years post PSC diagnosis in IBD patients^[48-51].

Despite these recommended guidelines, research show that scheduled colonoscopy is rarely performed in UC-PSC patients. A Canadian study followed up IBD-PSC patients for five years, and found that only 36% of the expected annual surveillance colonoscopies were conducted. 33% of patients did not undergo a single colonoscopy, and 11% of patients developed colorectal dysplasia or CRC during the follow-up period^[52]. Another study of 771 patients with an ≥ 8 years history of UC found the prevalence of annual surveillance amongst UC-PSC patients to be 38.5%, higher than that of the total study population (24.6%)^[53]. A recent study from the Mayo Clinic showed that the rate of colorectal neoplasm (dysplasia and carcinoma) discovery within two years of diagnosis of coexisting IBD and PSC (21.5 per 100 patients) was similar to rate of discovery within eight to ten years (20.4

per 100 patients)^[54]. This finding supports current guidelines for annual colonoscopic surveillance for IBD-PSC patients, starting from when concurrent PSC and IBD are diagnosed.

Rationale is lacking for the benefit of annual surveillance in IBD-PSC patients in the form of grade A supporting evidence, and current guidelines have been mainly based on expert opinion and retrospective studies. Little research has been done in the form of controlled trials to compare the rate of CRC diagnosis and prognosis between patients that undergo routine colonoscopy and those that do not. Data are unavailable that surveillance offers any prevention against CRC development or reduction in CRC mortality.

MECHANISMS OF PATHOGENESIS

Currently, the pathogenic mechanisms for the increased risk of CRC in UC-PSC patients remain unknown. One hypothesis suggests bile acids as the key culprit. PSC and other cholestatic conditions typically exhibit impaired hepatic excretion of bile acids, which may result in colonic build-up of secondary bile acids^[55]. Bile acids have long been suspected as a carcinogen in human gastrointestinal cancers. Studies in animal models have shown secondary bile acids to cause DNA damage and promote cell mutation^[56]. The observed increase in prevalence of CRC in the right proximal colon, where secondary bile acid concentrations are the highest suggests the role of bile acid in carcinogenesis^[25-27]. The strongest evidence for this hypothesis comes from the beneficial role of UDCA. Ursodeoxycholic acid modifies the bile acid pool to reduce levels of the secondary bile acid deoxycholic acid, thereby purportedly reducing the carcinogenic potential of bile acid^[57]. However, the preventative role of UDCA remains controversial, and similarly the role of bile acids in the development of CRC is also still up for debate.

Long-standing inflammation is a recognised risk factor in CRC development in IBD patients^[4]. Studies have shown that coexisting IBD in PSC patients often exhibit milder clinical courses. Patients often require less use of steroids, immunomodulators and surgery, and have reduced disease activity or even asymptomatic disease^[22,58]. Primary sclerosing cholangitis may be associated with a milder subclinical IBD for many years before diagnosis^[4,19] and hence have had longer disease duration than apparent, increasing their risk of CRC development and requiring surveillance to commence immediately upon diagnosis of PSC^[4].

CONCLUSION

Recent evidence increasingly points to PSC as a significant risk factor in the development of CRC in patients with concomitant IBD. Data suggest that the risk of CRC development can reach up to 30% at 20 years after diagnosis of concurrent IBD and PSC. PSC may be an important risk factor for CRC in different populations

worldwide. The mechanism for this increase in risk is still unclear. Various methods to prevent CRC development have been extensively investigated. The efficacy of UDCA remains controversial, and more longer term randomised placebo-controlled trials are needed. Liver transplantation does not halt the development of CRC, although there is not enough evidence to suggest that it is associated with increased incidence of CRC. Patients with concurrent PSC and IBD should be educated about the risk of CRC, and while routine colonoscopic surveillance should be performed, more high-level evidence is required to support the benefits of the procedure.

While many new developments have taken place in the last decade, the pathogenesis and optimal management of CRC development in IBD-PSC patients remain unclear. Further research in these directions will lead to better insight into the relationship between IBD, PSC and CRC.

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Treatment of Crohn's disease in pregnant women: Drug and multidisciplinary approaches

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Core tip: Patients should be encouraged to postpone conception until their Crohn's disease (CD) is in remission. Monitoring of nutritional status remains important in patients with small bowel CD; folic acid, vitamin D and vitamin B12 may all need to be supplemented. Most drug treatments are safe in pregnancy, based on observational data, including 5-aminosalicylic acid, thiopurines, anti-tumor necrosis factor, and anti-integrins. Methotrexate should be avoided due to its teratogenicity. Cesarean section is only indicated from a CD perspective in women with active perianal disease at the time of delivery; all others can have a normal vaginal delivery.

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Abstract

Inflammatory bowel disease affects a substantial number of women in their reproductive years. Pregnancy presents a number of challenges for clinicians and patients; the health of the baby needs to be balanced with the need to maintain remission in the mother. Historically, treatments for Crohn's disease (CD) were often discontinued during the pregnancy, or nursing period, due to concerns about teratogenicity. Fortunately, observational data has reported the relative safety of many agents used to treat CD, including 5-aminosalicylic acid, thiopurines, and tumor necrosis factor. Data on the long-term development outcomes of children exposed to these therapies *in utero* are still limited. It is most important that physicians educate the patient regarding the optimal time to conceive, discuss the possible risks, and together decide on the best management strategy.

INTRODUCTION

In recent years, great advances have been made in the management of inflammatory bowel diseases (IBD)^[1]. Nevertheless, many questions arise for physicians and patients when women with IBD consider pregnancy. In this situation, an understanding of the nutritional, pharmacological and diagnostic considerations is important for treating physicians to minimize harm to the fetus, while ensuring the mother's disease remains in remission^[2,3].

FERTILITY IN CROHN'S DISEASE

Fertility issues, risk of the disease in off-spring, and the impact of both the disease and medication on mother

Table 1 Food and Drug Administration drug administration categories for the use of medications in pregnancy

Category	Observations
A	Controlled studies both in humans and in animals have shown that there is no risk during the first trimester and the possibility of fetal harm is remote
B	Studies in animals have shown no risk to the fetus. However, no controlled studies have been carried out in pregnant women. In addition, studies in animals have revealed adverse effects which were not confirmed in pregnant women in the first trimester
C	There is no record of controlled studies in humans. Studies in animals have shown adverse effects. Moreover, studies in humans and animals showing that the benefit may outweigh the risk have not been validated
D	Evidence of risk for the fetus
X	Studies in animals and humans have shown fetal abnormalities, so these drugs are contraindicated

and child can all create fear in many patients wanting to have a child^[2]. Some factors may influence the fertility rate in patients with Crohn's disease (CD); these include active disease and/or malnutrition, and drug-induced oligospermia in male partners^[4]. Active CD in the colon or the terminal ileum or even surgery such as proctocolectomy with ileoanal anastomosis (colitis) have been associated with lower fertility rates^[4-6]. The psychological burden of disease may also play a role; in perianal CD, low fertility has been attributed to dyspareunia, decreased libido and depression in some women^[7].

Sulfasalazine is associated with infertility in men; in 80% of cases sperm motility is reduced and morphology is changed. Such effects are not reversible with the administration of folic acid, but they may reverse two months after the end of the sulfasalazine treatment^[8,9]. In contrast to these data, mezalazine, which is also a 5-ASA, and immunosuppressants such as azathioprine, do not appear to affect spermiogenesis in patients^[10].

EFFECTS OF INFLAMMATORY DISEASE ON PREGNANCY AND PREGNANCY EFFECTS ON INFLAMMATORY DISEASE

Even though most pregnant women with IBD may be classified as having high-risk pregnancies, the course of the disease in this group usually does not present major complications. The rate of premature births in this specific group is frequently double that in women without inflammatory disease^[3]. The risk of congenital malformation in the general population ranges from 1% to 4.8% and there is no current evidence of an increased risk for CD patients^[11]. Conversely, miscarriage is more frequent in women with IBD (above 35%), especially in those with active disease. The natural risk of fetal loss after 16 wk is approximately 1%, which is similar to the risk of healthy women^[12]. Some studies have shown that women with active CD are more likely to bear children with low birth weight (less than 2500 g)^[13].

The influence of pregnancy on the course of the disease is closely related to the disease status at the time of delivery *i.e.*, active or inactive. This status will determine the behavior of the disease itself, the clinical course, and the response to drug therapy^[7]. Some studies show that 70% of women with active disease have worsening or persisting symptoms during pregnancy, whereas the

risk of relapse in women who do not have active disease at conception is similar to women who are not pregnant^[14-25]. A recent meta-analysis of these studies concluded that the risk of active disease during pregnancy is higher in women who conceive when their disease is active^[26].

THERAPEUTIC DRUGS AND THEIR SAFETY DURING PREGNANCY AND BREASTFEEDING

Studies conducted in the last decades with women who underwent treatment during their pregnancy provides reassuring data for the specialist as well as for the patient both during the pregnancy and while breastfeeding. The Food and Drug Administration (FDA) classification for drugs according to their known or potential teratogenicity is reviewed in Table 1. Table 2 shows the safety of medications commonly prescribed for IBD during pregnancy.

Sulfasalazine and 5-aminosalicylic acid (category B)

5-aminosalicylic acid (5-ASA) is considered safe up to doses of 3 mg/d. Above this dose, the risk is considered uncertain^[8-10]. Studies show that sulfasalazine and 5-ASA in doses below 3 g/d do not increase the risk of congenital malformation, premature birth and miscarriage in patients with CD or ulcerative colitis (UC). A post-marketing study showed that of 55 pregnant women who used mezalazine in doses of 1.6 to 4 g/d, three had fetal malformations, however, these data were not different from those found in the general population, which suggests that there is no greater risk of malformations with mezalazine use^[9].

Azathioprine and 6-mercaptopurine (category D)

Thiopurines (azathioprine and mercaptopurine) both cross the placental barrier and can be identified in the umbilical cord blood, however, serum level in the baby is not significant. Animal studies showed the occurrence of cleft palate and skeletal and urogenital abnormalities in rats, and historical retrospective studies associated thiopurines with teratogenic effects in 5% of cases and the risk of preterm birth in 3%, in addition to the effects of low fetal weight and myelotoxicity^[4].

More recent observational studies did not observe a

Table 2 Safety of medications prescribed for inflammatory bowel disease during pregnancy

Safe to use when indicated	Limited data but used when clinically indicated	Contraindicated
Mesalamine	Olsalazine	Methotrexate
Sulfasalazine	AZA/6 MP	Thalidomide
Balsalazide	Ciprofloxacin	
Corticosteroids	Metronidazole	
TPN	Biologics	
Loperamide	Cyclosporine	

TPN: Total parenteral nutrition; AZA: Azathioprine MP: Mercaptopurine.

higher risk of these events in women with IBD. A recent prospective study of 30 children, performed by de Meij *et al.*^[15], evaluated the effect of azathioprine on the uterus in relation to quality of life, psychosocial development and an increased risk of infection, and showed that this drug did not directly influence these factors when compared to children who had not undergone this therapy. The American Academy of Pediatrics recommends that breastfeeding mothers should not take immunosuppressants, as these drugs induce immunosuppression in children^[16,22]. Most studies report that the most common adverse effect in pregnant women is related to low weight and miscarriage^[4].

Antibiotics

Metronidazole and ciprofloxacin are often administered in the treatment of patients with IBD, especially perianal CD. Metronidazole is classified as Category B, and short-term use (7-10 d) is considered safe in pregnancy. In contrast, extended use in the third month of pregnancy has been associated with fetal cleft palate and cleft lip, and therefore prolonged use during pregnancy is contraindicated^[4]. Ciprofloxacin is Category C, as quinolones act on the cartilage and in humans they can cause arthropathy and skeletal abnormalities of the fetus^[27]. For this reason, they are not recommended for children under 18 or for pregnant or breastfeeding women.

Corticosteroids (category C)

Corticosteroids (prednisolone) cross the placental barrier, however, they represent a very small risk when used in the first trimester of pregnancy. Studies carried out in animals have shown that these drugs may increase the risk of cleft palate and cleft lip when administered in the first trimester^[25]. Glucocorticoids should be administered with care and both blood pressure and blood glucose should be monitored due to their ability to induce gestational hypertension, diabetes, membrane rupture and preterm delivery.

Cyclosporine/tacrolimus (category C)

Cyclosporine and tacrolimus are both calcineurin inhibitors occasionally used in the management of CD. Both are category C, and can be employed in the treatment of fulminant colitis; their teratogenic action has not yet

been proven. Doses above 25 mg/kg per day can induce renal damage in the fetus in animals and their use in humans requires serum monitoring of renal function and blood pressure because both drugs cross the human placenta, however, there are conflicting reports on this point^[26].

Thalidomide (category X)

Thalidomide is rated as category X (FDA) for pregnant women due to its potential teratogenic effects. It is contraindicated in this population.

Methotrexate (category X)

This drug has also been classified as category X. It is clearly teratogenic and should not be considered for use in pregnant women and in women who want to conceive. Patients who are taking this medication should be instructed to delay conception for three to six months after its cessation. It can cause growth retardation and even mental retardation, among other effects.

Infliximab (category B)

Infliximab is a chimeric used in the treatment of CD and UC. It is known to cross the placental barrier after the second trimester, similar to all IgGs. Maternal and embryonic toxicity and increased teratogenicity have not been observed. Infliximab can be detected in high concentrations in the newborn up to 6 mo after delivery, but the clinical significance of this finding is unknown^[19]. Caution with any type of live vaccine in this group of infants during the first 6 mo is necessary, particularly if the infant received anti-tumor necrosis factors (TNFs) during gestation. There were no lethal cases of TB in three-month-old children who received BCG^[19].

Adalimumab (category B)

Considered by the FDA to be a category B drug, adalimumab has been approved for CD in induction, remission and maintenance phases. It exhibits similar behavior to infliximab, also crossing the placental barrier in the third month of pregnancy. There are few data on its use by pregnant women, and related birth defects have been reported, however, further studies are needed. Waage *et al.*^[20] conducted a review study of 126 women who had been subjected to treatment with adalimumab and no increased risk of congenital malformation was observed. Recent studies have indicated dose adjustments during pregnancy to reduce maternal exposure. It has been specifically recommended that the last dose should be given between 34 and 36 wk of gestation^[16].

Certolizumab pegol (category B)

Certolizumab is a Fab fragment of a monoclonal antibody linked to a polyethylene glycol chain. It is used during CD in remission and maintenance and it is known to cross the placental barrier throughout the pregnancy at a low level. In a recent study (PIANO), no increased risks in pregnant women administered certolizumab were ob-

Table 3 Classification of the drugs concerning the fetal risk according to Food and Drug Administration

Drugs	Recommendation
Adalimumab	Pregnancy (low risk)
Category B	Breastfeeding (probably compatible)
Azathioprine/6-mercaptopurina	Pregnancy (low risk) when used in low doses and as mono-therapy
Category D	Breastfeeding (it is recommended to breastfeed 4 h after taking the drug)
Balsalazide	Pregnancy (low risk)
Category B	Breastfeeding (probably compatible)
Certolizumab	Pregnancy (low risk)
Category B	Breastfeeding (probably compatible)
Ciprofloxacin	Pregnancy (not recommended due to skeletal muscular dysfunction)
Category C	Breastfeeding (compatible)
Corticosteroids	Pregnancy (risk of adrenal insufficiency, premature rupture of membrane, in the first trimester although there is little risk of cleft palate)
Category C	Breastfeeding (probably compatible)
Cyclosporine	Pregnancy (no congenital abnormalities have been noticed)
Category C	Breastfeeding (contraindicated)
Infliximab	Gestation (low risk when administered as mono-therapy) (increased risk of infection when used in combination with azathioprine)
Category B	Breastfeeding (probably compatible)
Mezalazine	Pregnancy (asacol showed low risk of teratogenicity in animal models)
Category B	Breastfeeding (both probably compatible)
Asacol (category C)	
Methotrexate	Contraindicated in both conditions
Category X	
Metronidazole	Pregnancy (used in the first trimester increases the risk of cleft palate)
Category B	Breastfeeding (toxic)
Olsalazine category (C)	Pregnancy (limited risk)
	Breastfeeding (probably compatible)
Rifaximin	Pregnancy (animal studies show teratogenicity)
Category C	Lactation (its safety is unknown)
Sulfasalazine	Pregnancy (low risk if administered in conjunction with folic acid)
Category B	Breastfeeding (probably compatible)
Tacrolimus	Pregnancy (no increased risk described)
Category C	Breastfeeding (contraindicated)
Thalidomide	Contraindicated in both conditions
Category X	

served^[21]. In addition, when breast milk was analyzed, it was noted that from 3 to 6 d post-birth, serum levels of the drug were not detected. Thus, it seems that this drug is safe in this phase^[21].

Golimumab

Golimumab is a completely human monoclonal antibody which aims to block anti-TNF. It is administered subcutaneously and was approved in May, 2013 by the FDA for the treatment of severe ulcerative colitis. To date, there are no reports on the use of this drug in pregnant women^[24].

Natalizumab (category C)

Natalizumab was recently approved for induction and maintenance treatment of CD in patients who do not respond to therapy with anti-TNF- α . Individual studies are necessary to prove that its use can be recommended for pregnant women. In a recent study with natalizumab, no increase in abnormalities were noted in pregnant women who had received the drug. A similar result was found in the PIANO study^[22]. However, most studies did not consider the drug to be safe enough to be used

during pregnancy; thus, it is contraindicated^[23].

DRUGS AND THEIR SAFETY DURING PREGNANCY AND BREASTFEEDING

It is important to bear in mind which drugs can be used during pregnancy and lactation. These drugs are shown in Table 3. In general, 5-ASA derivatives are considered safe (EL 3b, RGB) as well as corticosteroids (I.4, RGC). A low concentration of these steroids has been noted in breast milk. To minimize the effects of these drugs it has been suggested that mothers can breastfeed 4 h after their ingestion. The thiopurines are excreted in small amounts in milk, and are also considered safe. However, more studies are needed to fully confirm the safety of these drugs (EL 4, RGC). All anti-TNFs are also excreted in small amounts in the milk and there are few studies regarding the effects on children who are or were breastfed and consequently received these medications (EL5, RGC). Metronidazole and ciprofloxacin are also excreted in breast milk and are not considered appropriate during breastfeeding as their safety is unknown and these agents should, if possible, be avoided. Studies on tacrolimus

are limited and its safety is unconfirmed. Drugs such as thalidomide, methotrexate and cyclosporine are contraindicated as they have been found in breast milk and are consequently considered unsafe.

ENDOSCOPIC METHODS DURING PREGNANCY

Endoscopy, colonoscopy, retosigmoidoscopy and cholangiography are considered safe during pregnancy according to ECCO Statement 7G (EL4, RGC). However, caution is required in relation to these procedures and there must be a strong indication for these procedures to be carried out in the second trimester (EL5, RGD). Techniques for hemostasis are safe, but should be performed with caution (EL3, RGC).

RISK OF THROMBOEMBOLISM IN HOSPITALIZED PREGNANT WOMEN

Many studies have considered the increased risk of venous thromboembolism in pregnant women. This increased risk was noted in the first six weeks of the postnatal period, and is even higher in pregnant women with inflammatory bowel disease. The use of low-molecular-weight heparin is considered important to prevent this event and should be considered especially in women who have been or will be hospitalized (EL3 RGB).

CONCLUSION

Due to the current knowledge on inflammatory bowel disease, it is thought that the majority of drugs administered during pregnancy are safe for both the mother and the fetus. However, guidance in this group of patients (mothers-to-be) and control of disease activity before conception are essential for the prevention of miscarriage or premature birth. Most drugs are also safe for breastfeeding.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Approaches to improve quality of care in inflammatory bowel diseases

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Quality

Core tip: There is growing recognition in the variation and lack of quality of care in medicine, including the care of patients with inflammatory bowel disease. We review the existing literature on approaches to quality improvement and their potential application and barriers when applied to inflammatory bowel disease care.

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Abstract

Studies across medical disciplines have shown gaps in the care recommended in evidence based guidelines and the care actually delivered. Quality improvement projects using systematic audit and feedback interventions such as quality measures, will become increasingly important tools to address these gaps in care. These gaps are also apparent in the care of patients with inflammatory bowel disease. Multiple organizations, including the American Gastroenterology Association and the Crohn's and Colitis Foundation of America, have developed programs designed to implement quality measures to improve the care of inflammatory bowel disease (IBD) patients. Early results show promise of improving quality, but numerous barriers remain. Gastroenterologists need to be aware of these processes to provide the highest care possible to patients with IBD. We review the existing literature on approaches to quality improvement and their potential application and barriers when applied to IBD care.

QUALITY OF CARE AND USE OF QUALITY MEASURES

Quality in medical care has been defined by the Institute of Medicine as "the degree to which health services for individuals and populations increase the likelihood of desired health outcomes and are consistent with current professional knowledge"^[1]. Another description of quality care has also been called the triple aim, which is summarized as improving the experience of care, improving the health of populations and reducing per capita costs of healthcare^[2]. The current healthcare system is undergoing a shift from a fee for service model to a performance based model. This change will occur over the next few years and will likely change how we practice medicine. Integral to this shift from a fee for service to a performance based system is the concept of quality. To this end, different systems have been developed to improve quality and quantify that improvement.

Clinical practice guidelines are one of the current methods of improving the quality of care. They are developed through review of the literature and evidence based consensus, yet many of these recommendations have not translated into clinical practice. Despite development and distribution of numerous guidelines, gaps persist between recommended care and provided care. McGlynn *et al*^[3] evaluated a broad sampling of the United States population by randomly sampling patients, as part of the Community Tracking Study. Patients were sampled by telephone for any healthcare encounters in the preceding 2 years and then the investigators reviewed their medical charts for performance of specific quality measures. They found that 54.9% of quality measures were being met and little difference existed between provision of acute care, preventative care or chronic disease care. Similarly, gaps have been observed in specialty care. Calvin *et al*^[4] reviewed the compliance of physicians with prescription of beta blockers and angiotensin-converting-enzyme inhibitors or angiotensin receptor blocker's according to evidence based guidelines to patients with congestive heart failure. They found that only 63% of physicians were compliant with these guidelines and interestingly 37% of patients were noncompliant with evidence based prescription medication. These results highlight the obstacles to delivery of quality care, which requires the participation of the physician and the patient. Kanwal *et al*^[5] examined the adherence, to quality measures for ascites and cirrhosis among practitioners in the VA healthcare system. Though variation existed for individual quality measures, they found only 33.2% of patients received all recommended care. Absence of comorbid conditions, treatment by a gastroenterologist and care received at an academically affiliated hospital were correlated with higher rate of adherence to quality measures. These studies showed the limitation of passive guideline distribution to effect patient care and that more targeted interventions are needed to improve the quality of patient care.

METHODS TO IMPROVE QUALITY OF CARE

Audit and feedback systems are among the most effective interventions for quality improvement. In audit and feedback interventions, a provider's performance is measured and the results of their performance are shown to the provider with the intention of highlighting areas of deficiency and encouraging a change in practice. A systematic review of audit and feedback studies showed modest gains when feedback was provided in a timely manner and in written format^[6]. Audit and feedback is one of the underlying principles behind quality and performance measures. Quality measures are sets of quantifiable processes and outcomes defined by evidence based medicine and expert consensus to reflect high quality care for a specific disease. In contrast to clinical practice guidelines, which provide only passive dissemination of recommendations, quality and practice measures incorporate audit

and feedback to the provider, which may include external or financial incentives for adherence. However, audit and feedback interventions are limited by the type of data readily available for auditing. Current quality measures are therefore limited to processes or outcomes with available administrative or billing data, which may fail to capture the breadth of quality of care provided. Audit using manual chart review are more comprehensive, however are too time consuming to be used in routine practice. Use of standardized templates embedded in electronic medical records or use of information retrieval techniques from plain text medical records, such as natural language processing, are being developed. These approaches may expand the ability to quantify processes and outcomes beyond administrative data.

To have meaningful and long-term impact in clinical outcomes, quality measures need to be systematically integrated in clinical care, involving multidisciplinary teams and open access to share best practices. One approach to implement quality measures is the Donabedian model of structure, process, and outcome improve quality of care^[7]. Structure involves identifying the practice or system level factors that may influence how care is delivered. These include organizational structure (multi-disciplinary models), support staff numbers and qualifications and certifications for excellence in disease care^[8]. A process is defined as the actions required to move through the defined health care structure, including how patients interact with clinic staff and how diagnostic tests are performed. Outcomes are the effects of the health care delivered and may be either process measures (*i.e.*, if a vaccination was administered), or outcome measure (*i.e.*, colon cancer). Consideration of all of these components will identify not only what needs to be measured, as in quality measures, but what the components of a high quality care organization will require and how patients interact with that organization.

Systematic integration of quality measures has been used effectively in other disciplines of medicine to improve patient care. The Cystic Fibrosis Foundation's Quality Care Initiative provides an example of a successful intervention using quality measures in a chronic disease with a meaningful improvement in clinical outcomes. The initiative began as a collection of over 100 centers that provide cystic fibrosis (CF) care. After developing quality measures, which included weight, forced vital capacity and mortality they implemented a transparent system of audit and feedback. Outcomes were shared across all participating centers through regular meetings to review these outcomes and processes that result in improved outcomes. Since it was created, they have managed to improve the median age of death among CF patients from 27 years to 37 years^[9]. In cardiology, the Northern New England Cardiovascular Disease Study Group, which is a collaboration of multiple sites, has worked to improve several cardiovascular disease outcomes. Over a 2 year period, they achieved a 24% reduction of in-hospital mortality related to coronary artery bypass grafting^[9]. Taking these

examples, we can see how influencing particular processes in a collaborative and continuous way is able to affect meaningful clinical outcomes.

QUALITY OF CARE IN INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is very similar to the previously mentioned diseases of coronary artery disease, CHF, cirrhosis and CF, in that it is a chronic medical condition with a growing impact on the healthcare system. Kappelman *et al*^[10] examined the annual cost of IBD by review of administrative claims data. Their results showed Crohn's disease and ulcerative colitis (UC) mean annual costs were \$8265 and \$5066, respectively. When examined based on costs incurred inpatient, outpatient or medications related, costs were relatively equally distributed.

Despite numerous guideline publications addressing IBD care, gaps remain in the care provided and recommended. Reddy *et al*^[11] examined this gap in IBD patient care by reviewing the medical charts of patients referred to their tertiary referral center. They found that patients with active disease were receiving suboptimal dosing of current medications (64% for mesalamine) or were given medications, such as steroids, for prolonged periods of time without attempting steroid sparing agents (77%). Wagnon *et al*^[12] also showed, through a mailed survey study, that the screening and treatment for osteoporosis was highly variable between high (52%) and low volume centers (16%). In a retrospective study of adherence to colorectal cancer screening in ulcerative colitis patients, Velayos *et al*^[13] found that only approximately 25% of patients received adequate screening. These observations show that gaps in IBD care exist and implementation of quality improvement in IBD projects could have a meaningful impact.

QUALITY OF CARE INTERVENTIONS IN IBD

One of the first attempts to adopt quality improvement projects in IBD started in the United Kingdom after an audit in 2006 showed variation in the quality of care delivered to IBD patients. This group developed a set of quality measures addressing both structural measures and process measures centered on delivery of better patient care^[9]. The Improve Care Now (ICN) consortium in the United States is a collaboration between pediatric gastroenterology centers to develop quality measures regarding IBD. Prospective data is aggregated in a central database and used to generate weekly audit and feedback reports for the participating centers. These reports are reviewed by the sites and modifications are made to processes to improve outcomes. Involvement in this program has resulted in an increase in remission rates from 55% to 75% over the past few years^[9].

In Sweden, Rejler *et al*^[14] developed a quality improvement framework, consisting of demographic data, disease

characteristics, prior surgeries and medications, quality of life and measures to assess time from referral to scheduled appointment with a specialist, to a local community to measure its ability to record pertinent patient data. They found a lower prevalence of anemia than expected, a lower prevalence of surgeries than expected and overall good access to care. Parker *et al*^[15] measured the effect of a simple vaccination questionnaire prior to a clinic visit on the quality metric of vaccination compliance. They found significant improvement in the compliance with influenza vaccine before (54%) and after implementation of the questionnaire (81%).

In adult gastroenterology practice, the American Gastroenterology Association (AGA), in association with the American College of Gastroenterology and Crohn's and Colitis Foundation of America, has worked to develop a set of quality measures for IBD. The IBD measures (Tables 1 and 2) have been developed through collaboration of the AGA and the Crohn's and Colitis Foundation of America (CCFA) through two separate panels. The IBD performance measures are now part of the Centers for Medicare and Medicaid Services pay for performance program, Physician Quality Report Service (PQRS)^[16]. As physicians submit data to this system, they are currently eligible for reimbursement incentives and beginning in 2015 penalties will be applied to reimbursement if not meeting particular standards^[9]. This vividly shows the shift towards performance based care, which will shape the near future of our healthcare system. The CCFA has developed a separate IBD quality measure set using RAND/DELPHI panel consensus and reflect expert consensus of the available literature for the specific purpose of quality improvement rather than financial incentives^[17].

The PQRS IBD measure set has also been adopted by the Bridges to Excellence (BTE) program, which is being implemented through the Digestive Health Recognition Program by the AGA. BTE works by providers selecting 25 consecutive patients seen within a 12-mo period who have an IBD diagnosis. From each chart, specific metrics must be extracted, entered through a web portal and submitted to a third party for adjudication. Each metric is weighted and if a provider achieves a threshold number of points, they will be awarded BTE recognition for 2 years^[9]. Many third party payers are reviewing this system and considering incorporating this into incentives for reimbursement.

FUTURE DIRECTIONS AND POTENTIAL BARRIERS

While the quality measures for adults with IBD are a first step towards improving care, systemic and multidisciplinary approaches will be needed to affect meaningful and durable practice change. The CCFA, in collaboration with leaders from the ICN, have started a feasibility study to develop such a project. This pilot will be performed in both academic and community practices and is designed to study the best ways to implement IBD quality measures

Table 1 American gastroenterology association inflammatory bowel disease performance measures^[16]

Performance measures	
IBD characteristics	
	Type
	Anatomic location
	Activity
Preventive care	
	Corticosteroid sparing therapy
	Bone loss assessment
	Influenza immunization
	Pneumococcal immunization
	Smoking screening and cessation assessment
Testing	
	Latent tuberculosis testing prior to anti-TNF therapy
	Assessment for Hepatitis B prior to anti-TNF therapy
Inpatient care	
	<i>Clostridium difficile</i> testing in patients with new onset diarrhea
	Venous thromboembolism prophylaxis

IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor.

in adult gastrointestinal practices. As in the ICN, this project will involve individual reporting of quality measure and sharing of knowledge between sites to improve the overall care delivered to adult patients with IBD.

However several barriers to implement quality improvement in IBD still exist. Although success in improvement of remission rates have been observed in ICN, the structure and processes of care may not translate into adult practices. For example, in contrast to pediatric care of IBD or CF, care for adults with IBD is decentralized with many patients receiving care from non-referral centers. The differences in practice patterns between providers in academic and community gastroenterologists have been previously described in IBD. Esrailian *et al*^[18] performed a survey study comparing diagnostic and treatment decisions for IBD among community and academic-center affiliated gastroenterologists. They mailed surveys with clinical vignettes and asked for structured responses. They found overall agreement between groups regarding diagnostic measures, but found variation in treatments recommended both between and within groups. This suggests that heterogeneity exists both between community and academic groups and within these groups regarding therapeutic options. Spiegel *et al*^[19] also examined this issue in a national survey study comparing the responses of community gastroenterologists and UC experts. They found dramatic differences regarding both diagnostic evaluation and treatment decisions, though the vignettes were developed to address controversial issues in UC management. Other related, but distinct issues, are the differences between care endpoints in pediatric and adult practices. While nutrition is important in adult patients with IBD, outcomes assess growth and height may be less relevant in adults. These are some of the limitations to be addressed prior to wide adoption of quality improvement projects can occur in adult gastroenterology practices.

Table 2 Crohn's and Colitis Foundation of America inflammatory bowel disease process measures^[17]

Process measures	
Treatment	
	If anti-TNF therapy is considered, then test for tuberculosis with skin testing or interferon gamma release assay
	If anti-TNF therapy is considered, then assess for latent hepatitis B virus
	Consider steroid sparing agent, if steroids needed at 10 mg (of more) daily for > 16 wk
	Test for <i>Clostridium difficile</i> if a patient presents with new symptoms of diarrhea
	If planning to start 6-mercaptopurine or azathioprine, then test for thiopurine methyltransferase and dose accordingly
	If a patient is hospitalized with severe colitis and does not improve after 3 d of IV steroids, flexible sigmoidoscopy with biopsies should be performed to check for CMV infection and surgery consulted
Surveillance	
	If a patient with UC has low grade dysplasia in flat mucosa, then procto-colectomy or repeat surveillance in 6 mo should be offered
	If a patient has extensive UC or Crohn's disease involving the colon for 8-10 yr, then surveillance colonoscopy should be performed every 1-3 yr
Health maintenance	
	If a patient is on immunosuppressive therapy, then vaccinations for influenza and pneumococcus should be offered, as well as education regarding avoidance of live vaccines
	If a patient has Crohn's disease, smoking status should be assessed and smoking cessation recommended

TNF: Tumor necrosis factor; UC: Ulcerative colitis.

Another limitation to wide spread adoption of quality improvement interventions will be the time and financial constraints placed on physicians to implement the proposed quality improvement systems. Currently, physicians are faced with many pressures for their time and the possible addition of new processes could lead to unexpected adverse outcomes such as patient selection or new deficiencies in care areas not addressed in quality measures. A related issue involves the cost of establishing this system and the structure needed to provide continuous quality improvement. Illustrating this, the ICN network has shown an improved outcome of remission after implementing many process measures, but the program remains expensive and difficult to continue funding^[9]. Potential means to address these barriers are through innovations in bioinformatics. Development of integrated templates with automated data extraction of relevant clinical data into databases or information retrieval from plain text in EMR notes *via* natural language processing are possible means of accurately and efficiently gathering the data necessary for audit and feedback. Further work in these areas is required.

CONCLUSION

The system of quality measures and measurement of outcomes in IBD suggests many benefits regarding out-

comes. Several quality improvement programs have been established in IBD to develop the fundamental concepts and strategies for future quality improvement initiatives. Much has been learned from studying other chronic conditions, including CF and cardiac diseases, which can be applied to future quality initiatives within the field of IBD. Limitations remain to developing these systems and their implementation, and ongoing studies are needed to identify which outcomes should be used and how to accurately and efficiently provide feedback to providers. Overall, the healthcare system is moving towards a performance based model and as gastroenterologists we should be leading the development of potential measures and appropriate outcomes to help deliver the best care possible to our patients.

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Epidemiology, demographic characteristics and prognostic predictors of ulcerative colitis

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ful in identifying patients who tend to have more severe evolution of the disease and a poor prognosis. In this group of patients, better clinical surveillance and more intensive therapy may change the natural course of the disease. The aim of this article was to review the epidemiology and demographic characteristics of UC and the factors that may be associated with its clinical prognosis.

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Key words: Ulcerative colitis; Incidence; Prevalence; Risk factors; Predictive factors

Core tip: Ulcerative colitis has gained importance over the past few decades due to its increasing incidence rate worldwide. This condition is a chronic disease that affects quality of life, and it can lead to death if not treated properly. Over the past few decades, advances in treatment have provided benefits for patients, including a reduction in mortality. Due to phenotypic variability, different therapeutic modalities may be used. It is important to recognize the factors associated with a more severe clinical course so that clinical decisions can be made as early as possible.

Abstract

Ulcerative colitis (UC) is a chronic disease characterized by diffuse inflammation of the mucosa of the colon and rectum. The hallmark clinical symptom of UC is bloody diarrhea. The clinical course is marked by exacerbations and remissions, which may occur spontaneously or in response to treatment changes or intercurrent illnesses. UC is most commonly diagnosed in late adolescence or early adulthood, but it can occur at any age. The incidence of UC has increased worldwide over recent decades, especially in developing nations. In contrast, during this period, therapeutic advances have improved the life expectancy of patients, and there has been a decrease in the mortality rate over time. It is important to emphasize that there is considerable variability in the phenotypic presentation of UC. Within this context, certain clinical and demographic characteristics are use-

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INTRODUCTION

Ulcerative colitis (UC) is a chronic disease that is characterized by diffuse inflammation of the rectal and colonic mucosa. UC involves the rectum in 95% of cases and may be extended continuously and circumferentially to more proximal parts of the large intestine. The clas-

sic clinical symptom of UC is the presence of bloody diarrhea. The clinical course is characterized by periods of remission and exacerbation, which may occur either spontaneously or in response to treatment^[1].

The incidence of inflammatory bowel disease (IBD) has increased in several regions of the world in recent decades, especially in developing nations. Evidence indicates that there is an interaction between genetic and environmental factors in the etiology of the disease^[2]. Several studies have shown that certain clinical and demographic characteristics may be associated with different phenotypes and a poor prognosis in UC.

The identification of patients who tend to have more severe evolution of UC is important so that intensive treatments may be started earlier, with the goal of reducing complications and mortality.

The aim of this article was to review the epidemiology and demographic characteristics of UC and the factors that have been associated with poor prognostic outcomes.

EPIDEMIOLOGY AND DEMOGRAPHIC CHARACTERISTICS

Incidence and prevalence

The occurrence of UC worldwide has increased over the past few years. In contrast to the developed countries of North America and Western Europe, where the incidence of UC has plateaued or even decreased^[3,4], publications show that the number of cases has increased in developing countries, such as those in Latin America, Asia and Eastern Europe^[5-7]. Despite the increased incidence in these places, there are still differences in UC incidence and prevalence in different regions of the world. The incidence rate of UC may vary from 0.5 to 31.5 per 100000 people each year, depending on the studied population^[8].

The prevalence is lower in developing countries. In Asian populations, for example, the prevalence ranges from 5.3 to 63.6 per 100000 people^[9,10], whereas in North America, it ranges from 37.5 to 238 per 100000 people^[11]. In addition to the gradient between the occurrence of UC in the West and in Asian countries, it has been noted that in Europe, although there are exceptions, there is also a geographical gradient for the incidence of IBD, with higher rates in the north and a lower frequency in the south^[12]. Similarly, Sonnenberg *et al.*^[13] suggested a greater frequency of IBD in the northern United States compared with the south of the country. In Latin America, the prevalence of UC also appears to be variable. A study conducted in Puerto Rico suggested that the prevalence of UC is 12.53 cases per 100000 people^[5]. Victoria *et al.*^[14] concluded that the prevalence of UC in a southeastern region of Brazil has been increasing in recent years and that during the period from 2001 to 2005, the prevalence was 14.81 cases per 100000 habitants.

Mortality

According to previous studies, the mortality rate of pa-

tients with UC was higher in the first half of the twentieth century. Beginning in the mid-1950s, despite the ongoing increase in incidence, it appears that there has been a drop in the number of deaths, possibly due to the usage of corticosteroids and sulfasalazine and the optimization of surgical techniques^[15]. Despite this progress, data published in the 1980s showed that mortality in UC was higher than in the general population^[16]. Fortunately, overall, individuals with UC currently have mortality rates similar to or only slightly higher than the rate in the general population^[17,18]. This outcome may have been a consequence, among other factors, of the increased use of immunosuppressive therapy in recent years. However, when considering only the UC population, it is clear that mortality is higher in certain subgroups, and especially in newly diagnosed patients and in patients with extensive colitis^[17].

In meta-analyses of population-based cohort studies reported by Jess *et al.*^[20], five of 10 studies reported on UC-related mortality. The authors reported that among patients with UC, the mean percentage of deaths ascribed to UC itself was 17% (range 11% to 30%). In this subgroup of patients, the most common causes of death were colorectal cancer (CRC) (mean 37%, range 24% to 44%) and surgical or postoperative complications (mean 44%, range 17% to 100%). The remaining causes were primarily related to severe disease, *i.e.*, toxic megacolon, intestinal perforation, intestinal infarction, myocardial infarction secondary to anemia and end-stage liver disease due to primary sclerosing cholangitis. Additionally, the authors concluded that compared with general population, patients with UC are at an increased risk of dying of gastrointestinal diseases (OR = 2.5; 95%CI: 1.9-3.2; $P < 0.001$), nonalcoholic liver diseases (OR = 4.0; 95%CI: 2.5-6.5; $P < 0.001$), pulmonary embolisms (OR = 4.0; 95%CI: 1.5-8.7) and respiratory diseases (OR = 1.6; 95%CI: 1.3-2.0; $P < 0.001$), counterweighted by decreased mortality from pulmonary cancer (OR = 0.3; 95%CI: 0.1-0.9; $P = 0.04$). The overall mortality rate due to malignancy was not increased in UC, although there was a trend toward more frequent CRC (standardized mortality ratio = 1.9; 95%CI: 1.0-3.8; $P = 0.07$). The mortality rate due to hematological malignancy, and specifically leukemia and non-Hodgkin's lymphoma, were not increased^[17].

In an Australian study performed in 401 patients with UC, Selinger *et al.*^[19] concluded that the major causes of death were circulatory ($n = 42$; 44.2%), malignant ($n = 22$; 23.2%), digestive ($n = 11$; 11.6%) and respiratory ($n = 7$; 7.4%) diseases. Mortality from circulatory diseases was significantly more common in UC than in the general population [44.2% *vs* 33.8%; $P = 0.0001$; RR = 1.38 (95%CI: 1.11-1.72)], mainly due to ischemic heart disease [24.2% *vs* 15.9%; $P = 0.04$; RR = 2.04 (1.45-2.85)]. Death from cholangiocarcinoma occurred nearly 15 times more often among patients with UC than in the general population [4.3% *vs* 0.3%; $P < 0.0001$; RR = 14.28 (95%CI: 5.40-37.80)]. Fatal CRC was more frequent in UC than in

the general population [6.3% *vs* 2.7%; $P = 0.047$; $RR = 2.36$ (95%CI: 1.09-5.14)]^[19].

A Danish cohort study of 36080 patients with UC was performed by Jess *et al*^[20]. The authors reported that the overall risk of dying, compared with the risk in the general population, was high in the first year after UC diagnosis ($HR = 2.43$; 95%CI: 2.31-2.57) and then rapidly declined to a constant level of approximately 1.1 after 2 years. The risk of dying from infectious diseases, cardiovascular diseases, gastrointestinal disorders with or without the inclusion of IBD or CRC remained significantly increased in the long term, with HR estimates of 1.64 (95%CI: 1.24-2.17) for infectious diseases, 1.11 (95%CI: 1.01-1.21) for cardiovascular diseases, 1.26 (95%CI: 1.07-1.48) for gastrointestinal disorders other than UC and 1.47 (95%CI: 1.23-1.76) for CRC^[20].

Age

In recent decades, although there has been an increased incidence of UC in different age groups, the majority of patients with UC are in the age group of 30-40 years at diagnosis^[11]. It has been observed that the average age at diagnosis is usually slightly higher in Asian countries compared with Western countries^[21].

Certain publications indicate that a second incidence peak occurs in an older age group^[22,23]. A study by Souza *et al*^[24] in southeastern Brazil showed that there was a trend toward a second peak of new hospital admissions due to UC in the age group of 60-69 years old. However, there is no consensus in the literature regarding the existence of this second peak^[11].

Although UC is less common in children, recent studies have shown that the number of UC cases has increased in pediatric patients and adolescents. In Scotland, for example, in recent years, an increased incidence of UC in the age group under 16 years was observed. Comparing the periods 1990-1995 and 2003-2008, incidence rates increased from 1.59/100000 per year (95%CI: 1.28-1.94) to 2.06/100000 per year (95%CI: 1.70-2.47; $P = 0.023$)^[25]. In a recent publication, Pant *et al*^[26] demonstrated that in the United States, between 2000 and 2009, the number of hospitalizations of pediatric patients with UC increased from 4171 to 7127 per year. Lindberg *et al*^[27] suggested that the increased frequency of disease is more significant during puberty and adolescence than during childhood. The authors demonstrated that in recent years, although the incidence has increased in the age group of 11-15 years, this rate has remained stable in children under 10 years of age.

Gender

Most UC studies have shown a male predominance or an equal distribution between genders^[11,21]. In the past, Italian investigators have even suggested that polymorphisms in an enzyme involved in the signal transduction of insulin (cytosolic low-molecular-weight protein tyrosine phosphatase) could increase predisposition to the development of Crohn's disease (CD) in women and of

UC in men^[28]. However, this hypothesis was refuted in a more recent study by a group of Spanish investigators^[29]. Moreover, in contrast to the cited studies, other authors have found a high female incidence of the disease^[30]. In a recent publication describing 35404 cases of IBD, Betteridge *et al*^[31] reported a female predominance among patients with UC ($RR = 1.35$; 95%CI: 1.32-1.39).

PREDICTORS OF PROGNOSIS

Family history

Although a family history of UC is a risk factor for developing the disease, it does not seem to be a negative prognostic factor in patients with UC^[32,33].

In a prospective study, Henriksen *et al*^[34] found a 10.1% incidence of a family history of IBD among 454 individuals with UC. The authors concluded that although the group with a positive family history had further exacerbations of the disease in five years, there was no significant difference in drug therapy or indication for colectomy. In a retrospective study in 411 children with a diagnosis of IBD, 244 (59.4%) of whom had UC, Roma *et al*^[35] concluded that children with familial IBD had an earlier onset of disease compared with those with sporadic IBD. However, this difference had no significant impact on the clinical phenotypes, course and/or outcome of disease. Kuwahara *et al*^[36] obtained clinical data for 46114 UC cases. The present age and the age at disease onset were lower among patients with UC who had a family history than among those without a family history. However, the clinical course of patients with UC was not affected by family history.

Environmental factors

Patients with IBD have a genetic predisposition to the development of such diseases. It appears, however, that this predisposition alone is not sufficient for the onset of inflammation. The current belief is that genetically predisposed patients, when in contact with environmental factors, develop an inadequate immune response that ultimately causes inflammation of the gastrointestinal tract^[37]. Over time, many studies have attempted to support the hygiene hypothesis, although the data are conflicting, and well-designed prospective studies are needed^[38]. Until then, among the studied factors, only smoking and appendectomy have a well-defined influence on the risk of developing UC.

In contrast to what happens in CD, smoking is a protective factor against the development of UC. In a meta-analysis, Calkins concluded that the risk of non-smokers developing UC is approximately three times higher than that of smokers ($OR = 2.9$; 95%CI: 2.6-3.2)^[39]. Moreover, among patients with UC, those who do not smoke have a less favorable evolution of the disease over time^[40]. Aldhous *et al*^[41] concluded that five years after diagnosis, a decrease in the extent of UC was more common in smokers than in groups of former smokers and non-smokers. Smoking seems to be a protective factor against

colectomy (OR = 0.57; 95%CI: 0.38-0.85) and against the development of CRC (OR = 0.5; 95%CI: 0.2-0.9)^[42,43].

Appendectomy influences the emergence of IBD and is considered to be a risk factor for the development of CD^[44]. Conversely, in one of the first studies on this topic, Rutgeerts *et al.*^[45] concluded that appendectomy protects individuals against the emergence of UC. While studying the reason for this protection, Matsushita *et al.*^[46] suggested that the appendix plays an important role in the pathogenesis of UC. In a meta-analysis, Koutroubakis *et al.*^[47] concluded that performing an appendectomy reduced the chance of developing UC by 69% (OR = 0.31; 95%CI: 0.261-0.373). Certain studies have also suggested that appendectomy might influence the clinical course of UC by reducing the rate of recurrence of the disease and the need for immunosuppression and colectomy^[48-50]. However, the data are still conflicting, and the influence of appendectomy on the clinical course of UC needs to be further investigated in prospective studies.

The intestinal microbiota plays an important role in homeostasis and immune system functioning. Currently, it is believed that different environmental and genetic factors can promote changes in that microbiota. The formation of a pathogenic microflora in genetically predisposed individuals is associated with changes in epithelial function, dysregulation of the immune function of the gastrointestinal tract and persistent intestinal inflammation^[51]. Future studies may indicate which environmental factors are associated with the formation of an abnormal microbiota and whether these changes are only the cause or also be consequences of the changes introduced by IBD.

Nutritional factors

Knowledge about nutrition and nutritional status is not only important for the multidisciplinary team that treats patients with IBD but also for the patients themselves, who may have a wide range of questions regarding how nutrition affects their quality of life and the evolution of the disease^[52].

Nutritional deficiencies occur in 20% to 85% of patients with IBD, and protein-energy malnutrition is the most common^[53,54]. Although malnutrition is often related to CD, studies have shown similar rates of malnutrition between patients with CD and patients with UC^[55,56]. Malnutrition in these patients is associated with a poor quality of life and infection and with increased in-hospital mortality, length of stay and financial costs^[55,57,58]. It is noteworthy that body mass index (BMI), an indicator commonly used to define nutritional status, has several limitations^[59]. Jahnsen *et al.*^[60] reported that despite finding higher BMI values in patients with UC than in those with CD, lean body mass was not higher than in controls. Furthermore, a higher percentage of fat mass in patients with UC contributed to their increased weight. Therefore, BMI itself has adverse implications for the course of UC^[61,62].

Overweight or obese patients with UC have higher

rates of colectomy than do eutrophic patients^[63], an increased need for permanent ileostomy, longer hospital stays, higher rates of incisional hernia after ileoanal pouch anastomosis^[64,65], an increased risk of nonalcoholic fatty liver disease^[66] and thromboembolism^[67] and increased inflammation of the colon^[61]. Increased body weight has also been associated with an early loss of response to infliximab in IBD^[68]. In contrast, a study conducted by Markel *et al.*^[69] did not find any association between preoperative BMI and infectious complication in a post-operative wound in children with UC. In this group, 78% of patients were overweight (BMI > 25) at the time of surgery.

Age at diagnosis

The influence of age at diagnosis on the clinical course of UC is another controversial issue. Therefore, the Montreal classification of UC, in contrast to the classification of CD, does not include age at diagnosis as a criterion. So far, there is no convincing evidence that the creation of subgroups according to age at diagnosis would affect the clinical course of UC patients^[70].

Certain authors have evaluated the influence of age at diagnosis on the clinical evolution of patients with UC. Riegler *et al.*^[71] evaluated 1705 patients with UC in Italy and reported that younger patients had a greater need to use corticosteroids and a higher incidence of diarrhea and weight loss at diagnosis. An important study on the clinical course of UC (IBSEN study), conducted by Norwegian investigators, prospectively evaluated patients with UC for 10 years. Based on multivariate analysis, the authors found that the risk of colectomy in patients with an age at diagnosis of 50 years was 72% lower than in patients under 30 years old (HR = 0.28; 95%CI: 0.12-0.65)^[72]. The hypothesis of a milder and less aggressive clinical course in patients with an older age at diagnosis was corroborated by other recent studies^[73,74]. In contrast, other studies found no significant differences in prognosis when comparing groups with different ages at diagnosis^[75,76].

Extent of disease

According to the Montreal classification, based on location, UC can be classified into three different subtypes: proctitis (when inflammation is restricted to the mucosa of the rectum), left colitis (when inflammation extends beyond the rectum and to the splenic flexure) and extensive colitis (when inflammation reaches the mucosa proximal to the splenic flexure)^[70]. The most frequent location of UC may vary between different studies. Left colitis was most frequent in studies conducted in France (extensive colitis: 19.1%; left colitis: 52.3%; proctitis: 28.7%) and Portugal (extensive colitis: 28%; left colitis: 52%; proctitis: 21%)^[3,77]. In Asia, it appears that more patients have inflammatory processes limited to the mucosa of the rectum, as demonstrated by Ng *et al.*^[78] (extensive colitis: 31%; distal colitis: 32%; proctitis: 37%). In a study from southeastern Brazil that evaluated patients

diagnosed with UC in the period from 1980-1999 and in which the applied classification was different from the Montreal classification, the results were similar to those found in the Asian population. The occurrence of extensive colitis was detected in 28.3% of cases, whereas the frequencies of left and proctosigmoiditis were 29.7% and 32.4%, respectively^[24].

Furthermore, the extent of the disease in each patient is not fixed over time. In this context, it is important to emphasize that in patients with proctitis or left colitis, the inflammatory process may progress to more proximal segments of the colon. This phenomenon may require greater clinical surveillance and even changes in the therapeutic regimen. In a study by Alkim *et al.*^[79], for example, the progression of inflammation to the more proximal segments of the colon occurred in 16.1% of patients with proctitis. The percentage was even higher in a study by Park *et al.*^[80], which showed that in 5 and 10 years, there was proximal progression in 44.7% and 60% of patients, respectively, with proctitis or left colitis.

The extent of UC influences the clinical course and prognosis of the disease. Patients with extensive colitis are more likely to be subjected to more intensive therapies or even colectomy^[79,81]. In a retrospective study, Lee *et al.*^[82] demonstrated that the extent of disease is a factor that is independently associated with resistance to therapy with aminosalicylates (HR = 1.46; 95%CI: 1.01-2.10; $P = 0.04$). A Canadian study showed that left colitis (OR = 8.67; 95%CI: 1.79-41.87; $P < 0.001$) and extensive colitis (OR = 14.08; 95%CI: 3.12-63.60; $P < 0.001$) are also associated with more frequent usage of immunosuppressive drugs in patients with UC^[83].

Whereas patients with proctitis do not seem to be at an increased risk of developing CRC compared with the general population, individuals with left or extensive colitis have a higher propensity to evolve this complication^[84-87]. In a recent study, Manninen *et al.*^[85] concluded that patients with UC are at an increased risk of developing dysplasia and CRC compared with the general population (OR = 1.99; 95%CI: 1.14-3.25). The authors also noted that this risk is even higher in patients with extensive colitis (OR = 3.09; 95%CI: 1.5-5.75). Similar data were found in a recent meta-analysis, which described the risk of CRC in patients with UC as higher than in the general population (OR = 2.4; 95%CI: 2.1-2.7). Again, the risk was even higher in patients with extensive colitis (OR = 4.8; 95%CI: 3.9-5.9)^[86]. The most recent data on the evolution of CRC risk over time are conflicting. Whereas certain authors have shown a tendency to stabilize the risk^[88,89], Jess *et al.*^[90] showed a reduction in the period from 1979 to 2008. The authors suggested that this reduction occurred as a result of advances in therapeutic approaches to UC. Further studies are needed to evaluate the behavior of the risk of CRC in UC over time.

Disease activity

Clinical and laboratory characteristics that are consistent with greater disease activity at diagnosis have been shown

to be important prognostic factors in patients with UC. The stratification of disease activity is important both in the choice of treatment modalities and in the assessment of prognosis. Lennard-Jones *et al.*^[91] evaluated 56 variables among patients with active UC in 181 hospital admissions. The authors concluded that fever, tachycardia, the number of evacuations and serum albumin level are important predictors of treatment failure and the need for colectomy. Those patients who had persistent fever and more than 8 stools per day in the first 24 h of hospitalization had a 4-5 times higher chance of becoming refractory to medical treatment and needing surgery. Carbonnel *et al.*^[92] evaluated factors associated with the failure of intravenous steroid therapy in hospitalized patients with active UC. The authors found that the risk of treatment failure was higher in patients meeting the criteria for severe disease in the classification of Truelove and Witts (RR = 2.26; 95%CI: 1.11-4.61). Lau *et al.*^[83] reported that more than 10 evacuations per day and the presence of blood in the stool at diagnosis were associated with greater usage of immunosuppression in patients with UC. Furthermore, Travis *et al.*^[93] concluded that patients with severe colitis who were hospitalized for more than 3 d and who persisted in having more than 8 stools per day and an elevated C-reactive protein level (> 45 mg/L) had a higher chance of colectomy. In a cohort study that evaluated the prognosis of patients during the first ten years of the disease, Solberg *et al.*^[72] concluded that anemia, an elevated erythrocyte sedimentation rate and fever at diagnosis were associated with a greater need for colectomy over time.

Endoscopic exams allow the direct evaluation of lesions of the intestinal mucosa in patients with UC. The severity of the lesions usually reflects clinical disease activity and may help to identify patients who are more likely to evolve worse disease behavior over time^[92]. In a recent study, Canadian investigators found that the presence of moderate to severe endoscopic lesions was associated with an increased need for immunosuppression^[83]. Carbonnel *et al.*^[94] demonstrated that patients admitted with active UC who had extensive and deep ulcerations (severe endoscopic activity) had a greater need for colectomy than did those with moderate endoscopic activity. Rutter *et al.*^[95] demonstrated that there is a close relationship of the degree of endoscopic activity (OR = 2.54; 95%CI: 1.45-4.44; $P = 0.001$) and histological activity (OR = 5.13; 95%CI: 2.36-11.14; $P < 0.001$) with the risk of developing CRC in patients with UC.

Several studies have considered fecal markers, and especially lactoferrin and calprotectin, as useful tools for the assessment of disease activity in UC and the response to treatment^[96-98]. Recently, studies have shown that calprotectin and lactoferrin are also useful as predictors of clinical relapse in UC^[99,100]. A study conducted by British investigators, who evaluated patients admitted with severe UC who needed intravenous corticosteroids, showed that a higher average concentration of fecal calprotectin was associated with an increased rate of colectomy^[101].

Extraintestinal manifestations

The manifestations of UC may not be restricted to the colon and rectum. A variable percentage of patients may also have abnormalities in other organs and systems. Joint, skin, liver, eye and hematologic manifestations are common in patients with UC. Extraintestinal manifestations have been shown to be associated with a greater extent of disease and a worse prognosis^[102]. Lakatos *et al.*^[103] conducted a study with 619 patients with UC who were followed for 25 years. The authors concluded that the presence of extraintestinal manifestations was associated with greater extent of disease. In the pediatric population, a recent study of Gower-Rousseau *et al.*^[104] concluded that the presence of extraintestinal manifestations in pediatric patients with UC increases the risk of colectomy (HR = 3.4; 95%CI: 1.2-10.0; *P* = 0.02).

Individuals with UC and primary sclerosing cholangitis (PSC) have a different phenotypic behavior compared to patients with UC only. In this group of patients, the presence of PSC is associated with increased occurrence of extensive colitis and CRC^[105-107]. In a meta-analysis performed by Soetikno *et al.*^[108], the presence of PSC in patients with UC increased the risk of dysplasia and CRC (OR = 4.79; 95%CI: 3.58-6.41). Kornfeld *et al.*^[109] reported that the cumulative risk of individuals with UC and PSC to develop CRC was 25%, 33% and 40% at 10, 20 and 30 years from diagnosis of UC, respectively.

Serological markers

Anti-*Saccharomyces cerevisiae* antibody (ASCA) and perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA) were the first serological markers of IBD identified. The presence of a positive ASCA is more associated with CD, and p-ANCA is more associated with UC^[110]. Over the past several years, however, these two serum markers not only became markers used to differentiate both IBD forms but also appeared to have prognostic implications. There is evidence that the presence of ASCA is associated with severe and refractory CD, whereas p-ANCA positivity in patients with UC seems to be associated with resistance to treatment^[111,112]. This association has been reinforced by other recent publications. Ferrante *et al.*^[113] demonstrated that the usage of infliximab in patients with UC and p-ANCA+/ASCA- is more associated with a suboptimal early clinical response (OR = 0.40; 95%CI: 0.16-0.99; *P* = 0.049). In a study in a pediatric population with IBD, Dubinsky *et al.*^[114] concluded that the presence of a positive p-ANCA was independently associated with a primary non-response to anti-TNF α in UC patients.

Papp *et al.*^[115] found no association between ASCA, anti-laminaribioside carbohydrate antibody (ALCA), anti-chitobioside carbohydrate antibody (ACCA), anti-mannobioside carbohydrate antibody (AMCA) or anti-outer membrane porin C (anti-OmpC) and different phenotypes of UC.

There are only few studies on the role of antinuclear antibodies in UC. In a study in 97 patients with UC, Barahona-Garrido *et al.*^[116] concluded that the presence of

antinuclear antibodies is associated with an increased risk of steroid dependency (OR = 3.9; 95%CI: 1.4-14.9; *P* = 0.033).

CONCLUSION

The global prevalence and incidence of UC have increased in recent decades. The increase in the number of new cases has been more evident in developing countries. Nevertheless, the mortality rate of UC has decreased over time, and currently, mortality in patients with UC is similar to or slightly higher than that in the general population. Environmental factors appear to be associated with the pathogenesis of UC. Among these factors, smoking and appendectomy have been considered protective against the development of UC. Moreover, evidence suggests that smoking and appendectomy are associated with less severe forms of UC and seem to confer protection against colectomy. Inversely, a greater extent of disease and higher disease activity are associated with a worse prognosis. Additionally, one should be attentive to the occurrence of nutritional deficiencies and extraintestinal manifestations, especially PSC, as well as to the presence of positive p-ANCA. The influence of age at diagnosis on the clinical course of UC is controversial, and therefore, further studies are needed to better evaluate this issue. Thus, based on current knowledge, it appears that demographic and clinical characteristics are useful to identify patients who tend to have more severe evolution of the disease. Earlier identification may allow more intensive therapeutic measures to be adopted earlier in the management of such patients.

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Ulcerative colitis as a polymicrobial infection characterized by sustained broken mucus barrier

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thought to be the intestinal bacteria, gut mucus, and the mucosa-associated immune system.

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Core tip: Long-term or even life-long medication bothers patients with ulcerative colitis (UC). Existing treatment ignores the cause of UC, so establishing the etiology of UC is the key to resolving this problem. UC can be viewed as a polymicrobial infection that is characterized by a sustained broken mucus barrier with subsequent bacterial migration toward the mucosa and proliferation of complex bacterial biofilms on the epithelial surface. Regulation of mucus secretion and viscosity, suppression of bacterial biofilms, probiotics and immunostimulation should be increasingly considered to treat UC.

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Abstract

To reduce medication for patients with ulcerative colitis (UC), we need to establish the etiology of UC. The intestinal microbiota of patients with inflammatory bowel disease (IBD) has been shown to differ from that of healthy controls and abundant data indicate that it changes in both composition and localization. Small intestinal bacterial overgrowth is significantly higher in IBD patients compared with controls. Probiotics have been investigated for their capacity to reduce the severity of UC. The luminal surfaces of the gastrointestinal tract are covered by a mucus layer. This normally acts as a barrier that does not allow bacteria to reach the epithelial cells and thus limits the direct contact between the host and the bacteria. The mucus layer in the colon comprises an inner layer that is firmly adherent to the intestinal mucosa, and an outer layer that can be washed off with minimal rinsing. Some bacteria can dissolve the protective inner mucus layer. Defects in renewal and formation of the inner mucus layer allow bacteria to reach the epithelium and have implications for the causes of colitis. In this review, important elements of UC pathology are

INTRODUCTION

Ulcerative colitis (UC) belongs to a subgroup of inflammatory bowel diseases (IBDs), is characterized as chronic inflammation, and has become a global health threat^[1,2]. High disease-recurrence rates, long-term or even life-long medication bothers patients with UC^[3-5]. Existing treatment ignores the cause of UC, and is unable to cure UC, which is why patients need long-term medication; therefore, establishing the etiology of UC is the

Table 1 Interaction of mucus and bacteria

Effect of mucus on bacteria	Effect of bacteria on mucus
Limits the direct contact between the host and bacteria	Maintenance of the mucus layer is stimulated by bacterial fermentation products
Serves as a source of nutrients for bacterial growth	Mediate the expression of MUC2
Contributes to the selection of the species-specific colon flora	Dissolve the protective inner mucus layer
Contains several proteins that limit bacterial growth and penetration	

key to resolving this problem. Important elements of IBD pathology are thought to be genetics, the intestinal microbiome, the gut mucosa, and the mucosa-associated immune system^[6,7]. Studies using dextran sulfate sodium (DSS) models of colitis also suggest that the key contributors in disease pathogenesis include alteration in the mucosal barrier integrity and function^[8,9]. The human gastrointestinal tract is a vast surface inhabited by a complex and diverse community of micro-organisms^[10], and the intestinal mucus is an efficient system for protecting the epithelium from bacteria by promoting their clearance and separating them from the mucosal immune cells, thereby inhibiting inflammation and infection^[11]. UC is an immune-mediated disorder that results from an abnormal interaction between colonic bacteria and mucosal immune cells in a genetically susceptible host^[12]. In this review, UC is thought to be a polymicrobial infection characterized by sustained broken mucus barrier.

ROLE OF MUCUS AND BACTERIA IN UC

The gastrointestinal tract is covered by a layer of mucus that protects the epithelium from luminal antigens and provides lubrication to advance the bolus^[13]. A well-developed mucus barrier and not the epithelial cell layer is the first line of defense against a variety of enteric pathogens^[14,15]. Leukocytes migrate into and patrol within the mucus layer, executing the surveillance function without any collateral damage. The sticky outer mucus surface offers the opportunity for probiotic strains to grow and build protective interlaced layers, preventing bacterial accumulation and microcolony formation on the colorectal surface^[16,17]. Before bacteria can adhere and invade the mucosa, they must first traverse the mucus barrier^[17]. The inflammation takes place only after the mucus barrier is broken and the defense is overwhelmed. UC is caused by a weakening in gut barrier, mainly due to increased infiltration of gut bacteria and the resultant recruitment of neutrophils and formation of crypt abscess^[18]. Understanding the role of mucus and bacteria and their interaction will help us to establish the etiology of UC more clearly (Table 1).

Role of mucus in UC

Mucus production and secretion is a continuously ongoing process with a renewal of the inner protective mucus in the distal colon within an hour. Rapid renewal of the mucus barrier prevents microbial contact with the epithelial cells. Alteration of the adherent mucus barrier is a predisposing factor for early onset of epithelial cell dam-

age in DSS colitis^[19]. Sulfation of the mucins is significantly reduced in UC patients, and suggest that colonic mucins plays an important role in maintaining the normal physiological function of the colon and the possible role of mucus in the pathogenesis of UC^[20]. Phosphatidylcholine (PC) accounts for > 70% of total phospholipids within the intestinal mucus layer, and the mucus PC content is reduced by about 70% in UC^[21]. MUC2 is the major mucin in the large intestine^[22,23], which is secreted by goblet cells, and the expression of it correlated with the activity of disease and the extent of the inflammatory process in the large intestine^[24]. Individuals with UC have decreased numbers of goblet cells and reduced mucus thickness at presentation^[25], and goblet cell abnormalities play an etiological role in UC. DSS models of colitis were characterized by depletion of goblet cell and adherent mucin^[19]. In UC, the cooperation of aberrant expression of Hes1 and the disappearance of caudal type homeobox 2 (CDX2) caused Hath1 suppression, resulting in goblet cell depletion^[26], and the present study suggests that Hes1 is essential for Hath1 gene suppression via Notch signaling. Gerseemann *et al*^[27] also pointed that in UC, the protective mucus layer, acting as a physical and chemical barrier between the gut epithelium and the luminal microbes, is thinner and in part denuded as compared to controls, and this could be caused by a missing induction of the goblet cell differentiation factors Hath1 and KLF4, leading to immature goblet cells. This goblet cell differentiation in UC can lead to defects in renewal and formation of the inner mucus layer and may enable the luminal microbes to invade the mucosa and trigger the inflammation^[16,27,28]. So we can easily reach the conclusion that understanding the regulation of goblet cell differentiation and the intestinal mucus turnover and renewal of the inner protective mucus layer is important for novel ways to improve treatment of UC^[16,21].

Role of bacteria in UC

UC is a multifactorial disease that is dependent on host genetics, environment, immune response and intestinal microbiota. The dysregulation of the gut microbiota plays an important role in the pathogenesis of UC^[29]. The immunoregulatory function of the intestinal microbiota consists of priming the mucosal immune system and maintenance of intestinal epithelial homeostasis. Epithelial barrier dysfunction brings about increased bacterial translocation through the lamina propria^[30,31]. Ineffective bacterial clearance leads to excessive Toll-like receptor (TLR) stimulation, secretion of proinflammatory cytokines and activation of innate and T-cell-mediated

immune responses. TLR-2 can bind a wide range of ligands, including lipoteichoic acid from Gram-positive bacteria, bacterial lipopeptides and glycolipids, and fungal β glucan (zymosan). TLR-4 can bind lipopolysaccharide from Gram-negative bacteria. Flagellin has innate qualities through its repetitive structures that are able to bind TLR-5, and also is a polypeptide that is internalized, processed and presented by professional antigen-presenting cells (APCs). Thus, the earliest phases of an immune response are dependent upon the recognition and interpretation of the antigenic composition of the milieu by T cells and APCs as revealed by innate and adaptive immune responses. TLR-9 is able to recognize bacterial DNA^[32-34], and the stimulation of TLR-9 causes activation of nuclear factor- κ B signaling, and leads to immune response and mucosal inflammation. These features could help to explain the mechanism of UC.

Defects in renewal and formation of the inner mucus layer allow bacteria to reach the epithelium and have implications for the causes of colitis^[28]. Neutrophils and mononuclear cells infiltrate the lamina propria and activate nuclear factor- κ B translocation, which in turn increases proinflammatory cytokines such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor- α , and inhibits the production of anti-inflammatory cytokines such as IL-10^[35]. *Fusobacterium varium* (*F. varium*) was present in the colonic mucosa of a high proportion (84%) of UC patients^[36] and contribute to the clinical activity in UC^[37]. *L. crispatus* CCTCC M206119 strain is involved in exacerbation of intestinal inflammation in DSS-colitis mice, and may interact directly with colonic epithelial cells or lamina propria mononuclear cells after disruption of the mucosal barrier and balance of gut flora by DSS administration. *Campylobacter* spp.^[38], *Escherichia coli*^[39-41], *Enterobacteriaceae*^[42,43], and *Bacteroides ovatus*^[44] are also responsible for the induction of intestinal inflammation.

However, not all the bacteria promote inflammation; *Pediococcus acidilactici*^[45], *Lactobacillus* spp.^[45], and *Bacteroides* spp.^[46] show a variety of beneficial immunomodulatory effects in UC. Their products, rather than live bacteria, may be capable of inducing immunoregulatory effects, and may restore the dysregulated functions of immune cells^[47]. Some recent studies have demonstrated that TLR signaling in intestinal sites can also inhibit inflammatory responses and maintain colonic homeostasis^[48,49].

Effect of mucus layer on bacteria

The mammalian gastrointestinal tract harbors a vast microbial ecosystem, known as the microbiota. Gut microbiota includes around 1000 different species and > 15000 different strains of bacteria, for a total weight of about 1 kg. The luminal surfaces of the gastrointestinal tract are covered by a mucus layer composed mainly of mucins, which are high-molecular-weight glycoproteins characterized by extended serine, threonine, and proline-rich domains in the protein core^[50]. This layer is a biochemically complex medium, rich in carbohydrates, antimicrobial peptides and other proteins, as well as lipids and electro-

lytes^[51]. The inner mucus layer normally acts as a barrier that does not allow bacteria to reach the epithelial cells and thus limits the direct contact between the host and bacteria^[52]. The mucus layer covering the gastrointestinal tract also has been reported to serve as a source of nutrients for bacterial growth. Thus, its presence influences intestinal colonization by attracting bacteria that have the ability to survive and multiply within the mucus layer^[53,54]. We have also found that the numerous O-glycans on the MUC2 mucin serve as nutrients for the bacteria as well as attachment sites, and as such, probably contribute to the selection of the species-specific colon flora^[44]. Overproduction of MUC2 may alter adherence and invasion of *Shigella dysenteriae* into human colonic epithelial cells. At the same time, the mucus also contains several proteins that limit bacterial growth and penetration, such as the antibacterial proteins and IgA^[10,20]. These are important for the assembly and stability of the microbiota.

Effect of bacteria on mucus

The mucus barrier, however, can be compromised by environmental or genetic factors as well as specific pathogens such as *Serpulina*, *Fusobacterium*, *Enterobacteriaceae*, or *Gardnerella*. These bacteria can specifically form adherent biofilms on the epithelial surface, compromising the mucus barrier and allowing migration of other indigenous bacteria into the mucosa. The commensal bacteria in the colon live and thrive in the outer loose mucus layer, and can dissolve this layer^[55]. Nevertheless, the association of the microbiota with the mucus is not well understood and requires further investigation.

The importance of bacterial exposure to produce a functional mucus barrier is demonstrated by germ-free animals in which the inner mucus layer is thin^[56], but can be restored by exposure to bacterial components^[56]. Maintenance of the mucus layer is also known to be stimulated by bacterial fermentation products^[57]. In conclusion, the bacteria can influence mucus production^[56]. Proteins secreted by probiotic bacteria of antimicrobial substances can enhance the mucosal barrier function and compete with enteropathogens for adhesion sites^[58,59]. The composition of short-chain fatty acids in the intestine is determined by the composition of the microbiota, and butyrate can mediate MUC2 mRNA *via* activator protein-1 and acetylation/methylation of histones at the MUC2 promoter. The microbiota can also mediate MUC2 mRNA^[58], and MUC2 can potentially be modulated in several other ways either during infection, such as at the level of gene expression, or even at the level of secretion into the intestinal lumen. Each regulatory step may influence the biological function of MUC2, which in turn influences how the host responds to enteric pathogens^[16]. MUC2 is reportedly overexpressed in response to bacterial components, such as lipopolysaccharide or lipoteichoic acid, in cultured intestinal or airway epithelial cells and also bladder epithelial cells^[13,60].

Bacteria can also dissolve the protective inner mucus layer, potentially triggering colitis. MUC2 is the

major colonic secretory mucin. We found that bacteria can produce proteases capable of dissolving the inner protective mucus layer by specific cleavages in the MUC2 mucin and that this cleavage can be modulated by site-specific O-glycosylation. However, because of O-glycosylation, the mucin domains are highly resistant to proteases and are not expected to be cleaved by proteases^[52]. However, MUC2 glycosylation can still be metabolized by intestinal commensal or pathogenic bacteria, serving as an energy source, suggesting a role in intestinal microbiota selection^[20,61]. The 980-amino-acid-long C-terminal part of MUC2 has two cleavage sites. One is localized to the NR2QA sequence within the VWD4 domain where the cleavage site is surrounded by numerous cysteines that are involved in disulfide bond formation. The second cleavage site is localized prior to the first cysteine in the MUC2 C-terminal VWD4 domain. The enzyme secreted by *Entamoeba histolytica* can dissolve the guanidinium-chloride-insoluble mucus gel that we now know is the major constituent of the inner firm mucus layer^[10]. *Porphyromonas gingivalis* also secretes a protease as an active enzyme to cleave MUC2, and this enzyme was isolated and identified as Arg-gingipain B. *Citrobacter rodentium* colonizes the outer mucus layer in high numbers, lacks a functional flagellum and is thus non-motile, and therefore likely utilizes specific mucinases or glycosidases to digest mucin in order to overcome the mucus barrier^[16,20].

CHANGES OF BACTERIAS IN UC

The intestinal microbiota of IBD patients has been shown to differ from that of healthy controls; abundant data indicate that the microbiota in IBD patients changes in both composition and localization^[62,63], and the changes are not a product of colitis, which has previously been reported^[64]. These support an integrative view of microbial ecology relevant to IBD^[65], and butyrate-producing bacteria could be important to gut homeostasis^[66,67]. The diversity of fecal microbiota is significantly lower in UC patients. *Bacteroides*^[68], *Clostridium* subcluster XIVab^[66], *Lactobacillus* spp.^[67], *Akkermansia muciniphila*^[69] and *Clostridium leptum*^[70] are decreased in UC patients, and the number of *Enterococcus*^[68], *Escherichia coli*^[71], *Actinobacteria*^[72], *Proteobacteria*^[73] and *Campylobacter ureolyticus*^[73] are higher in UC patients than in healthy subjects. Sulfate-reducing bacterium levels are also raised in UC^[74], and are crucial for induction of DSS colitis in mice. Some research has proposed that *F. varium* might be one of the elusive pathogenic factors in UC^[6]. Data also showed that the amount and composition of bacteria clearly differed between the mucus layers in animals not treated with DSS, with significantly higher loads of bacteria in the outer mucus layer^[75], and *Lactobacillus crispatus* CCTCC M206119 strain is involved in exacerbation of intestinal inflammation in DSS-colitis mice^[76]. Recently, we also found that small intestinal bacterial overgrowth was significantly higher in IBD patients as compared to controls^[35]. Despite the requirement

of commensal bacteria for normal intestinal function, an abnormal host response to commensal bacteria has been implicated as a crucial factor in the pathogenesis of IBD^[77,78]. Recent research has shown that some commensal and pathogenic bacteria are closely related to UC, but it is difficult to draw a definitive conclusion in evaluating the role of microflora in pathogenesis of UC, and to find specific micro-organisms associated with the pathogenesis of UC.

USE OF PROBIOTICS IN UC

Bacteria are closely related to UC, and recently some studies have investigated the use of probiotics in UC^[47,79,80]. Probiotics contain viable organisms; sufficient amounts of which reach the intestine in an active state, thus exerting positive health effects^[81]. Their mechanisms of action are still unclear, but several have been postulated to contribute to the anti-inflammatory effect of probiotics in the gut, including competitive exclusion of pathogens. Probiotics may potentially alter the intestinal microbiome exogenously or provide an option to deliver microbial metabolic products to alter the chronicity of intestinal mucosal inflammation^[82]. Bifidobacteria and lactobacilli produce harmful substances for Gram-positive and Gram-negative bacteria, and they compete with pathogens (*i.e.*, *Clostridium*, *Bacteroidetes*, *Staphylococcus*, and *Enterobacter*) for cell adhesion^[83,84]. Production of antimicrobial agents (*e.g.*, IgA) and organic acids, modulation of lymphocyte and dendritic cell function^[85,86], enhancement of the epithelial barrier function, modulation of the membrane permeability and mucosal immune system, and keeping pathogens away from the intestinal mucosal surface are also included. Probiotics have been investigated for their capacity to reduce the severity of UC (Table 2). The efficacy of VSL#3 (*Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus*) in UC patients has also been demonstrated^[94-96]. Also, some natural anti-inflammatory effects have recently been shown for *Lactobacillus salivarius*, *L. plantarum*, *Lactobacillus casei* Shirota, *Lactobacillus reuteri* and *Bifidobacterium* based on experimental colitis models^[76,97-99].

CONCLUSION

UC is mainly due to increased infiltration of gut bacteria and the resultant recruitment of neutrophils and formation of crypt abscess^[18], and can be viewed as a polymicrobial infection that is characterized by a sustained broken mucus barrier with subsequent bacterial migration toward the mucosa and proliferation of complex bacterial biofilms on the epithelial surface. Regulation of the mucus secretion and viscosity, suppression of bacterial biofilms, probiotics and immunostimulation should be increasingly considered to treat UC and evaluated in the future^[17].

Table 2 Probiotics have undergone investigation for their capacity to reduce the severity of ulcerative colitis

Probiotics	Method	Conclusion
<i>B. infantis</i> 35624 ^[87]	Oral administration of <i>B. infantis</i> 35624 for 6-8 wk is taken by patients with ulcerative colitis	This microbe can reduce systemic pro-inflammatory biomarkers in UC
<i>L. reuteri</i> ATCC 55730 ^[88]	Mild to moderate UC were received an enema solution containing 10 (10) CFU of <i>L. reuteri</i> ATCC 55730 for 8 wk, in addition to oral mesalazine	In children with active distal ulcerative colitis, rectal infusion of <i>L. reuteri</i> is effective in improving mucosal inflammation and changing mucosal expression levels of some cytokines involved in the mechanisms of inflammatory bowel disease
<i>B. breve</i> strain ^[89]	Mild to moderate UC ingested 1 g of the probiotic powder [10 (9) CFU/g] three times a day, and 5.5 g of GOS once a day for one year	Administration of live <i>B. breve</i> strain Yakult and GOS can improve the clinical condition of patients with UC
<i>L. delbrueckii</i> and <i>L. fermentum</i> ^[90]	Mild to moderate UC were treated with sulfasalazine 2400 mg/d with a probiotic preparation (which contained powder with 10 (9) CFU of <i>L. delbrueckii</i> and <i>L. fermentum</i> , for eight consecutive weeks	Oral supplementation with probiotics could be helpful in maintaining remission and preventing relapse of UC
<i>L. casei</i> DG ^[91]	Mild left-sided UC were received oral 5-ASA and rectal <i>L. casei</i> DG	Manipulation of mucosal microbiota by <i>L. casei</i> DG and its effects on the mucosal immune system seem to be required to mediate the beneficial activities of probiotics in UC patients
<i>EcN</i> ^[92]	Moderate distal UC were randomly assigned to treatment with either 40, 20, or 10 mL enemas ($n = 24, 23, 23$) containing 10 (8) <i>EcN</i> /mL ($n = 20$). The study medication was taken once daily for 2, 4, 8 wk	<i>EcN</i> is a well tolerated treatment alternative in moderate distal UC
<i>B. longum</i> ^[93]	The probiotic group ingested one daily capsule consisting of <i>B. longum</i> 2 × 10 (9) CFU	Patients with UC on probiotic therapy experienced greater quality-of-life changes than before

B. Infantis: *Bifidobacterium infantis*; *L. Reuteri*: *Lactobacillus reuteri*; *B. Breve*: *Bifidobacterium breve*; *L. Delbrueckii*: *Lactobacillus delbrueckii*; *L. Fermentum*: *Lactobacillus fermentum*; *L. Casei*: *Lactobacillus casei*; *EcN*: *E. coli* Nissle; *B. Longum*: *Bifidobacterium longum*; CFU: Colony-forming units; GOS: Galacto-oligosaccharide; 5-ASA: 5-aminosalicylic acid; UC: Ulcerative colitis.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Intestinal antigen-presenting cells in mucosal immune homeostasis: Crosstalk between dendritic cells, macrophages and B-cells

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Here, we review the role of all three types of APC in intestinal immunity, both in the steady state and in inflammation, and how these cells interact with one another, as well as with the intestinal microenvironment, to shape mucosal immune responses. We describe mechanisms of maintaining intestinal immune tolerance in the steady state but also inappropriate responses of APC to components of the gut microbiota that contribute to pathology in IBD.

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Key words: Antigen presenting cells; Dendritic cells; Macrophages; B cells; Inflammatory bowel disease

Abstract

The intestinal immune system maintains a delicate balance between immunogenicity against invading pathogens and tolerance of the commensal microbiota. Inflammatory bowel disease (IBD) involves a breakdown in tolerance towards the microbiota. Dendritic cells (DC), macrophages (MΦ) and B-cells are known as professional antigen-presenting cells (APC) due to their specialization in presenting processed antigen to T-cells, and in turn shaping types of T-cell responses generated. Intestinal DC are migratory cells, unique in their ability to generate primary T-cell responses in mesenteric lymph nodes or Peyer's patches, whilst MΦ and B-cells contribute to polarization and differentiation of secondary T-cell responses in the gut lamina propria. The antigen-sampling function of gut DC and MΦ enables them to sample bacterial antigens from the gut lumen to determine types of T-cell responses generated. The primary function of intestinal B-cells involves their secretion of large amounts of immunoglobulin A, which in turn contributes to epithelial barrier function and limits immune responses towards to microbiota.

Core tip: The intestinal immune-system maintains a delicate balance between immunogenicity against invading pathogens and tolerance of the commensal microbiota. Inflammatory bowel disease involves a breakdown in tolerance towards the microbiota. Dendritic cells, macrophages and B-cells are known as professional antigen-presenting cells (APC) due to their specialization in presenting processed antigen to T-cells, and in turn shaping types of T-cell responses generated. Here, we present an updated knowledge toward the role of these APC in intestinal immunity, both in the steady state and in inflammation, and how they interact with one another and with the intestinal microenvironment to shape mucosal immune responses.

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INTRODUCTION

Dendritic cells (DC), macrophages (MΦ) and B-cells comprise heterogeneous populations of cells, known as “professional antigen-presenting cells (APC)” due to their specialization in antigen presentation. APC are critical for initiating, maintaining and shaping T-cell mediated immune responses. DC are unique in their ability to drive primary T-cell responses (reviewed in^[1,2]), but MΦ and B-cells can polarize effector T-cell responses^[3-5]. All three types of APC also have other critical roles in both innate and adaptive immunity; at intestinal sites, a combination of these functions and crosstalk between APCs enable them to be critical for maintenance of immune homeostasis in the gut.

The gastrointestinal tract is in contact with a huge amount of antigens, including a diverse commensal microbiota, food antigens and also potentially pathogenic microbes. As effector cells of both innate and adaptive immune responses, DC, MΦ and B-cells are central to not only maintaining protective immunity against pathogens but also preventing inflammatory intestinal immune responses against the microbiota and food antigens (tolerance). The microbiota is recognized by pattern recognition receptors (PRRs) on all three types of APC, including Toll-like receptors (TLRs; reviewed in^[6]). Similar effector functions to those involved in protective immunity against pathogens are engaged during inappropriate inflammatory responses against harmless antigens, such as those seen in inflammatory bowel disease (IBD). IBD, including Crohn's disease (CD) and ulcerative colitis (UC), is thought to result from a dysregulated intestinal immune response to the gut microbiota^[7] resulting in a breakdown in mucosal tolerance. Given the huge antigenic load in the normal healthy intestine, APC in the steady state maintain a tolerogenic or hyporesponsive state, giving these cells crucial roles in maintaining mucosal homeostasis. In this review, we discuss the different roles of the three “professional” APC: DC, MΦ and B-cells, in intestinal immune tolerance and inflammation, and how these APC interact with one other to shape their function and contribution to mucosal immune homeostasis.

ANTIGEN PRESENTING CELLS AND INTESTINAL HOMEOSTASIS

Dendritic cells

DC stimulate primary T-cell responses and determine whether these T-cell responses generated are immunogenic (*e.g.*, against invading pathogens) or tolerogenic (*e.g.*, against commensal bacterial antigens)^[1,2]. The primary function of intestinal DC is to transport antigens into secondary lymphoid tissue [mesenteric lymph nodes (MLN) and Peyer's patches (PP)] and subsequently generate antigen-specific intestinal T-cell responses. Intestinal DC from the gut lamina propria (LP) transport intestinal bacterial antigens into MLN^[8,9] and are essential for inducing oral tolerance to food antigens^[10].

Intestinal DC in the steady state are generally hyporesponsive^[11] and maintain immune tolerance in the gut by generation of tolerogenic T-cell responses^[12,13] towards food antigens and commensal bacteria, preventing unnecessary inflammation and hypersensitivity. Although distinguishing DC from MΦ in the gut can be difficult, intestinal DC in mice can be identified as CD11c^{hi} MHC Class II⁺ CX3CR1⁺ F4/80⁻ cells and further subdivided into DC subsets expressing combinations of CD11b, CD103 and CD8α^[14]. Although previous studies suggested a subset of DC exist expressing CX3CR1, the receptor for the chemokine fractalkine, mononuclear phagocytes in the gut mucosa expressing CX3CR1 also express the pan-MΦ marker F4/80 and tissue macrophage marker CD68^[15]. Subsequent studies confirmed that all CX3CR1^{hi} cells in the gut are indeed MΦ^[16]. DC can also be distinguished from MΦ in both mice and humans on the basis of CD64 expression, with DC being CD64⁻^[17].

Several intestinal DC subsets contribute to regulatory T-cell (T-reg) generation in mice. These include CD8α⁺ DC that promote T-reg generation in the presence of transforming growth factor (TGF)-β^[18,19], and CD103⁺ DC. CD103 (αE integrin) is expressed by the majority of DC in the murine intestinal LP^[20]; these DC are migratory and travel to the MLN *via* the afferent lymph^[21-23]. In the steady state, this constitutive migration of CD103⁺ DC from the LP to the MLN establishes T-cell responses specific for harmless luminal antigens, and is essential for the establishment of oral tolerance^[10,13,21,24]. The ability of CD103⁺ DC to synthesize retinoic acid (RA)^[25,26], which enhances generation of gut-homing T-reg at the expense of Th17 cells^[25-28], is one of the key mechanisms by which CD103⁺ DC participate in immune tolerance in the gut. Human studies indicate DC from MLN maintain some of the unique tolerogenic properties of murine intestinal CD103⁺ DC^[21,29]. Furthermore, CD103⁺ DC from the LP in both mice and humans express indoleamine 2,3-dioxygenase (IDO), an enzyme involved in the ability to drive T-reg development, is required for the establishment of immune tolerance in the gut^[30]. Plasmacytoid DC (pDC) are also key participants in oral tolerance^[31] likely to be due to their expression of IDO.

Intestinal CD103⁺ DC can be subdivided into two major subsets; CD103⁺CD11b⁺ and CD103⁺CD11b⁻ DC^[32]. CD103⁺CD11b⁺ DC stimulate Th17 and Th1 cell differentiation^[33,34], whilst CD103⁺CD11b⁻ DC can drive Th1 polarisation and IFNγ-production from CD8⁺ T-cells^[34,35]. However, other studies have shown both CD103⁺ subsets can generate T-reg responses^[36]. Interpretation of the regulatory function of these intestinal subsets is further complicated by the fact that mice lacking either CD103⁺CD11b⁺^[33,37] or CD103⁺CD11b⁻ DC^[38] have normal numbers of intestinal FoxP3⁺ T-reg. CD103⁺CD11b⁺ intestinal DC are also potent inducers of both Th17 and Th1 responses, even in the absence of overt stimulation^[35], and a subsequent study using comparative analysis of transcriptomes determined that CD103⁺Sirpα⁺ DC in the human gut are related to murine

CD103⁺CD11b⁻ DC (and human blood CD141⁺ DC), whilst human intestinal CD103⁺Sirpα⁺ DC were related to murine CD103⁺CD11b⁺ DC (and human blood CD1c⁺ DC). In this study, both these human intestinal DC subsets were able to induce Th17 cells, with only CD103⁺Sirpα⁺ supporting induction of T-reg^[39].

MΦ

Intestinal MΦ have various innate functions that enable them to contribute to both immune tolerance *via* selective inertia and contribute to protective immune responses and inflammation in other circumstances^[15]. Tissue MΦ do not usually migrate to lymphoid tissue, but can contribute to adaptive immune responses by presenting processed antigen to effector T-cells *in situ* in the LP^[3,4]. Although intestinal MΦ share expression of MHC Class II, CD11c and CD11b with DC, F4/80, CD68 and CD64 can be used to identify MΦ in the gut. It has also now evident that all CX3CR1^{hi} mononuclear phagocytes are MΦ^[16], although a subset of inflammatory, migratory CD103⁻ DC expressing intermediate levels of CX3CR1 has recently been identified^[23].

Resident intestinal MΦ express low levels of co-stimulatory molecules including CD80, CD86 and CD40^[34-38], and like intestinal DC, are hyporesponsive to stimulation by TLR ligands^[12,35,39,40] in the steady state. MΦ in the gut also contribute to maintaining intestinal immune tolerance by constitutively producing the anti-inflammatory cytokine interleukin (IL)-10^[39,40]. Perhaps the most striking role for intestinal MΦ in maintaining mucosal homeostasis is their role in generation and maintaining survival of T-reg. F4/80 knockout (KO) mice do not develop tolerance or antigen-specific CD8⁺ T-reg. normally after being fed soluble antigen^[41]. MΦ secretion of IL-10 plays a key role in maintaining FoxP3 expression on T-reg. under inflammatory conditions, essential for maintaining regulatory activity and suppressing colitis^[42]. Furthermore, tolerance induction following feeding with protein antigens in mice was associated with expansion and differentiation of FoxP3⁺ T-reg by IL-10-producing CX3CR1⁺ MΦ in the mucosal LP^[4].

Intestinal CX3CR1⁺ MΦ have recently been subcharacterised; CX3CR1^{hi} MΦ in the steady state represent regulatory MΦ that are resistant to TLR stimulation and produce IL-10 constitutively, whilst a smaller population of cells expressing intermediate levels of CX3CR1 represent cells partially differentiated from Ly6C⁺CCR2⁺ monocytes into regulatory CX3CR1^{hi} MΦ. These CX3CR1^{int} cells represent TLR-responsive, pro-inflammatory MΦ that accumulate during experimental colitis due to arrested differentiation^[16]. This study demonstrated that both resident regulatory MΦ and inflammatory MΦ at intestinal sites are both derived from the same (Ly6C⁺CCR2⁺) precursor, but are at different phases of differentiation.

B-cells

The role of B-cells in intestinal inflammation and im-

mune homeostasis have been underappreciated. B-cells perform several immunological functions; arguably their main function is antibody production, but B-cells also function as APC and secrete cytokines. At intestinal sites, B-cells follow a distinct differentiation pathway and are specialized in IgA production as differentiated plasma cells^[43]. Most intestinal plasma cells secrete IgA^[5]; in the gut lumen, secretory IgA (sIgA) acts as a barrier to protect the epithelium from pathogens. Within the gut lumen, sIgA interacts with intestinal antigens including the intestinal microbiota, food antigens and self antigens^[44]. In such a manner, sIgA limits access of intestinal antigens into the bloodstream, and is able to control the intestinal microbiota^[5]. The sIgA system in the gut is tightly integrated with both innate and adaptive immune mechanisms, contributing towards intestinal immune homeostasis. For example, sIgA can limit innate responses against commensal bacteria^[45] whilst also functioning to influence adaptive T-cell responses^[46]. Several regulatory compounds involved in intestinal tolerance also promote IgA secretion, including IL-10, TGFβ and RA^[43].

The antigen-presenting function of B-cells enables them to interact with T-cells directly to polarize effector T-cell responses^[47] (although they are not capable of inducing primary responses). Several disease models demonstrate that IL-10 produced by B-cells is important for the generation of mucosal T-reg^[48-51]. However, IL-10 produced by DC and MΦ is also important in T-reg generation^[42,52], and alone is not sufficient to induce T-reg. directly; cognate T-cell/B-cell interactions are also required, mediated by co-stimulatory molecules CD80 and CD86^[53,54].

IL-10-producing B-cells with suppressive capacity are known as regulatory B-cells (B-reg) and can suppress experimental colitis^[55-57]. A subset of B-reg can also produce regulatory cytokine TGFβ in response to antigenic stimulation^[58-60], demonstrating an important role for B-cells in avoiding inappropriate responses to the intestinal microbiota and food proteins. Indeed, functional impairment of this subset of TGFβ-producing B-cells is associated with food allergy pathogenesis^[58-60]. It has recently been demonstrated that a subpopulation of B-cells carries the integrin αβ6 (not endogenously expressed) which is able to convert latent TGFβ into its active form. These cells also expressed CX3CR1, had high levels of TGFβ, generated T-reg. suppressed T-cell activation and inhibited food allergy symptoms^[61]. This study suggests CX3CR1⁺ B-cells carrying αβ6 may represent the TGFβ-producing B-reg. described in the studies above.

INAPPROPRIATE ANTIGEN-PRESENTING CELL RESPONSES TO MICROBIOTA AND INFLAMMATION

Despite playing essential roles in intestinal immune tolerance, APC are likely to be of fundamental importance in the pathogenesis of T-cell mediated inflammation in the

gut; all APC can influence T-cell responses directly and can secrete both pro- and anti-inflammatory cytokines. IBD is thought to result from a dysregulated immune response and breakdown of tolerance to the gut microbiota^[7,62,63]. The intestinal microbiota is essential for development of colonic inflammation in most murine models of colitis^[64], although in the steady state the gut microbiota functions to reduce bacterial trafficking to MLN by mononuclear phagocytes to downregulate inflammatory responses and autoimmunity^[65]. Analysis of the intestinal microbiota of IBD patients demonstrates decreased biodiversity, with decreased proportions of Firmicutes but increased proportions of Gammaproteobacteria^[66]. It is currently unclear whether intestinal dysbiosis in IBD patients contributes to or is a consequence of inflammation but the interplay between the host and the microbiota actively shapes intestinal homeostasis and contributes to IBD pathology. This provides a role for all APC in IBD pathogenesis due to their bacterial recognition properties *via* PRR expression. The ability of DC and M Φ to sample antigens from the gut lumen, and ability of B-cells to produce sIgA that modify immune responses to luminal antigens suggests APC play important roles in dysregulated immunity in IBD. Expression of TLRs on DC^[67,68], M Φ ^[69-71] and B-cells^[72] are upregulated in animal models of colitis and human IBD, potentially contributing to enhanced or inappropriate responses to luminal bacterial antigens.

Intestinal dendritic cells in gut inflammation

As the only cells capable of driving primary T-cell responses^[73], intestinal DC would be expected to play an important role in T-cell dominated inflammatory diseases at intestinal sites, such as IBD. For example, animal models of colitis provide strong evidence that interactions between the intestinal microbiota and intestinal DC are essential for IBD pathogenesis^[63,74]. Activated DC accumulate throughout the LP and MLN in colitis^[75-77]; intestinal DC present during inflammation may be derived from newly recruited precursors^[78], although it is likely that tissue-resident *in situ* DC can also generate inflammatory responses. Therefore, intestinal DC are likely to play both protective and pathogenic roles in intestinal immunity, fitting with their functional plasticity in their ability to generate either inflammatory or tolerogenic immune responses. In murine DSS-induced colitis, DC ablation during DSS administration ameliorated disease manifestation, but colitis was exacerbated if DC were ablated before DSS treatment^[79]. In a T-cell transfer model of colitis induced by CD45RB^{hi}CD4⁺ T-cells, transplanted T-cells formed aggregates with sub-epithelial CD11c⁺ DC in the MLN^[80]. Furthermore, colitis was associated with increased CD11c⁺ DC in MLN, and blocking OX40-OX40L interactions between DC and T-cells prevented the development of colitis^[81]. In human IBD activated DC also accumulate at sites of intestinal inflammation^[82-84]. Some human studies have shown an increase in number and maturation of DC within inflamed IBD tissue^[85], but others suggest en-

hanced recruitment of immature DC into inflamed tissue associated with increased expression of chemokine CCL20 in the intestinal epithelium^[86]. CCL20 may therefore regulate attraction of DC (and T-cells) in IBD.

The specific microenvironment of the gut, including microbes, various types of intestinal cells such as epithelial cells, and active cellular mediators can dynamically shape the properties and functions of DC. For example, human blood DC express both skin and gut homing markers; however, they lost homing marker expression when cultured *in vitro*. Conditioning of human enriched blood DC with colonic biopsy extract induced a gut-homing phenotype and a homeostatic profile, mediated by retinoid acid and TGF β , respectively^[87]. In UC patients, circulating DC displayed a reduced stimulatory capacity for T cells and enhanced expression of skin-homing markers CLA and CCR4 on stimulated T cells that were negative for gut-homing marker β 7; and this dysregulation of DC could be partially restored by probiotic bacterial strain *Lactobacillus casei* Shirota^[88].

Bacterial recognition by dendritic cells and gut-homing

An important function of DC is their ability to imprint homing properties on T-cells and B-cells, in order to localize immune responses to a particular tissue^[89-92]. Murine intestinal DC specifically imprint gut-homing molecules α 4 β 7 and CCR9 on T-cells and B-cells from different sources, thus targeting lymphocytes to intestinal tissue^[89-91]; this gut-specific imprinting property of DC is confined to the CD103⁺ “tolerogenic” intestinal DC subset. T-cells imprinted with gut-homing capacities in such a manner are T-regs, linking gut-homing with intestinal immune tolerance. Furthermore, CD103⁺ DC induce B-cell class switching to IgA-producing cells with known tolerogenic properties, alongside imprinting gut-homing properties^[20,93,94]. These functional properties of CD103⁺ DC are dependent on RA and TGF β ^[39,95,96]. A loss of CD103⁺ DC from inflamed murine intestine has been reported^[97,98], suggesting the increased DC infiltrates in colitis/IBD represent alternative inflammatory DC subsets. Further evidence to link gut-homing and intestinal immune tolerance was provided by studies showing that expression of gut-homing markers α 4 β 7 and CCR9 on T-cells is essential for induction of oral immune tolerance in mice^[99]. Both CCR9 and α 4 β 7 expression on DC also confer tolerogenic properties^[98,100,101], and a loss of α 4 β 7⁺ DC impairs induction of IL-10-producing T-regs and accelerates T-cell mediated colitis^[98].

MyD88 is an essential intracellular signaling adapter for most TLR signals^[102], which are induced in APC following bacterial recognition. MyD88-dependent TLR signaling in DC specifically enables them to imprint gut-specific homing properties *via* an increased RA synthesizing capacity^[103]. In this study, TLR stimulation was sufficient to educate extraintestinal DC with gut-homing imprinting capacity providing a crucial role for the microbiota in shaping gut DC function in intestinal homeostasis. Although it is unclear whether the intestinal dysbiosis in

IBD patients is a cause or a result of intestinal inflammation^[66], it is likely that such alterations in bacterial populations will have knock on effects on gut DC function in tolerance and immunity, perhaps disrupting the delicate balance that is maintained in the healthy gut and further contributing to pathology.

Intestinal macrophages and B-cells in inflammation

Intestinal MΦ and B-cells can contribute to adaptive immune responses by presenting processed antigen to effector T-cells *in situ* in the LP^[3,4,47], and polarizing effector T-cell responses, thereby providing a role for MΦ and B-cells in IBD pathogenesis. Properties of intestinal MΦ are strikingly different in inflammation compared with the steady state. Under inflammatory conditions, MΦ infiltration into intestinal sites of inflammation occurs; these MΦ express high levels of TLRs, co-stimulatory and inflammatory receptors^[35,37,69,104], and produce large quantities of pro-inflammatory cytokines and mediators^[104-108]. Inflammatory MΦ in the murine intestine are derived from Ly6C⁺ monocytes; CCR2 is essential for recruitment of these Ly6C⁺ monocytes to sites of inflammation and in an inflammatory context, these monocytes upregulate expression of TLR2 and NOD2, suggesting an enhanced responses to the microbiota and bacterial products^[70]. Although this study actually suggests these inflammatory monocyte precursors develop into CX3CR1⁺ regulatory DC, concurrent studies have shown Ly6C⁺CCR2⁺ monocytes differentiate into regulatory CX3CR1^{hi} MΦ but that in colitis, there is accumulation of inflammatory Ly6C⁺CX3CR1⁺, TLR-responsive, pro-inflammatory semi-mature MΦ arising from arrested differentiation^[16]. The upregulated expression of TLRs on both inflammatory monocytes and macrophages during intestinal inflammation strongly suggests interactions of these cells with the microbiota and bacterial products play a key role in IBD.

B-cells can also present antigen to effector T-cells in the LP, but their unique antibody-secreting function enables B-cells to directly control the intestinal microbiota *via* sIgA^[5]. Due to the regulatory function of IgA in contributing to maintenance of epithelial barrier function^[109,110], aberrations in the mucosal IgA system are likely to be part of IBD pathogenesis. However, IgA has been reported to play a pathogenic role in the pathogenesis of other gut-based inflammatory disorders, including Coeliac disease^[111]. Although production of sIgA directly links B-cells to immune regulation and homeostasis, their role in IBD is unclear due to their other cytokine- and chemokine-producing functions. Intestinal B-cells in IBD are increased^[112] and highly activated, producing chemokines including Eotaxin-1, leading to acute eosinophilia^[113]. Furthermore, a loss of anti-inflammatory IL-10 production by B-cells in IBD has been reported^[114] alongside unusual B-cell morphology^[115], changes in DNA methylation^[116] and other B-cell gene alterations^[117]. However, the role of B-cell in IBD pathogenesis is unclear and warrants further investigation.

ANTIGEN-PRESENTING CELL CROSSTALK

As established, DC, MΦ and B-cells have critical roles both in maintaining mucosal immune tolerance to the gut microbiota and food antigens, but also in driving inflammatory responses that can be protective in healthy individuals, but detrimental in IBD. Although each type of APC exhibits unique functions allowing them to participate in gut immunity, with knock on effects on adaptive T-cell responses, APC can also interact with one another to directly shape immune responses generated. DC in particular are at the centre of virtually all multi-cellular signalling networks underlying intestinal immune homeostasis^[118].

Activation of intestinal B-cells by dendritic cells and macrophages

The interplay between innate immunity and B-cells at the intestinal mucosal interface play a key role in maintaining mucosal immune homeostasis^[119]. Although DC are usually described for their ability to prime T-cell responses, DC can also directly activate B-cells^[120,121], present unprocessed antigens to B-cells^[122,123] and influence the differentiation and survival of antibody-secreting cells^[124]. Intestinal DC release powerful B-cell stimulating factors including BAFF and APRIL^[125,126]; pDC in particular induce IgA production by B-cells in the gut, independently of T-cells, in this manner. In the steady state, this process is dependent on stromal cell-derived type I IFN signalling^[127]. Macrophages also release BAFF at levels sufficient to potently induce B-cell proliferation^[128]. BAFF and APRIL promote survival of B-cells and plasma cells but also activate IgA production^[129-134]. Intestinal DC and MΦ also produce IgA-inducing cytokines including IL-10 and TGFβ^[39,118,135]. Some intestinal DC can also produce IL-6, which is a cytokine implicated in the differentiation of IgA class-switched B-cells into IgA class-switched plasma cells^[124,136]. Alongside directly shaping B-cell responses, gut DC induce expression of gut-homing receptors CCR9 and α4β7 by B-cells^[94].

RA is essential for induction of gut-homing receptors on B-cells^[94], as is the case for T-cells^[137]. The IgA promoting effects of gut DC is at least partially dependent on RA and TGFβ^[94,138,139] and intestinal MΦ also secrete RA/TGFβ^[39]. A key question that remains unanswered is whether intestinal MΦ can also imprint tissue homing properties on B-cells and directly promote IgA class switching, properties that are both dependent on the presence of RA. Secretion of RA by intestinal DC and MΦ is dependent upon their expression of retinal dehydrogenases, which are critical for RA synthesis^[25,137-139]. The expression of these enzymes by intestinal DC is restricted to CD103⁺ DC^[25,140], and CD103⁺ intestinal DC do indeed promote IgA synthesis by gut-homing B-cells^[138]. Studies have since demonstrated that follicular DC also promote IgA generation in the gut in response to bacterial stimuli, and express key factors for B-cell mi-

Table 1 Summary of information available regarding classification of gut antigen-presenting cells, primary functions of gut antigen-presenting cells and their effects on T-cells, tolerogenic and inflammatory properties of antigen-presenting cells at intestinal sites, and effects of immunomodulation of gut antigen-presenting cells by the microbiota

Classification	Dendritic cells	Macrophages	B-cells
	Mice: CD11c ^{hi} MHC Class II ⁺ CX3CR1 ^{med} F4/80 ⁻ CD64 ⁻ . Subsets based on CD11b, CD103 and CD8α expression Humans: HLA-DR ⁺ Lineage cocktail (CD3/CD14/CD16/CD19/CD34) ⁻ . Subsets based on CD103, CD11c, CD1c, CD141 and CD123 expression	Mice: CD11c ⁺ MHC Class II ⁺ F4/80 ⁺ CD68 ⁺ CD64 ⁺ CX3CR1 ^{hi} Subsets based on levels of expression of CX3CR1. Ly6C ⁺ in inflammation Humans: HLA-DR ⁺ CD11c ⁺ CD64 ⁺ CD68 ⁺ Some CX3CR1 expression	CD19 ⁺ CD20 ⁺ CD79a ⁺ Immature B-cells: ⁺ CD20 Plasma (antibody secreting) cells: CD38 ⁺ CD138 ⁺
Primary function	Antigen sampling Migration to secondary lymphoid tissue and stimulation of naïve T-cells to generate primary T-cell responses	Antigen sampling Phagocytosis of apoptotic cells, bactericidal activity, production of anti-inflammatory IL-10	Antibody secretion as differentiated plasma cells (mainly IgA in the gut)
Effects on T-cells	Determine whether primary T-cell responses are immunogenic or tolerogenic, imprint gut-homing receptors on T-cells during stimulation	Contribute to effector T-cell responses <i>in situ</i> in the lamina propria, including expansion and differentiation of T-regs <i>via</i> IL-10 production	Contribute to effector T-cell responses <i>in situ</i> in lamina propria and also induce differentiation of T-regs <i>via</i> both IL-10 production and direct interaction
Tolerogenic properties/subsets	CD103 ⁺ CD11b ⁻ DC generate RA for T-regs/IgA secretion by B-cells, and imprinting gut-homing properties on lymphocytes. CD8α ⁺ DC and pDC generate T-reg Gut DC in general are hyporesponsive to TLR stimulation	CX3CR1 ^{hi} MΦ produce IL-10 critical for T-reg generation Hyporesponsive to TLR stimulation	IgA production limits immune responses against commensal bacteria Regulatory B-cells produce IL-10, induce differentiation of T-regs and also produce TGFβ
Inflammatory properties/subsets	TLR ^{hi} gut DC in IBD likely to contribute to enhanced inappropriate responses to the microbiota Infiltrates of CD103 ⁺ DC in inflammation CD103 ⁺ CD11b ⁺ can polarise inflammatory Th17 responses	TLR ^{hi} MΦ in colitis and IBD also likely to contribute to enhanced inappropriate responses to the microbiota Ly6C ⁺ CX3CR1 ⁺ inflammatory macrophages arise from arrested differentiation in colitis	TLR ^{hi} B-cells enhanced in IBD Eotaxin-1 producing B-cells enhanced in IBD CD15 ⁺ B-cells with functional surface IgM enhanced in IBD
Modulation by gut microbiota	Direct modulation by microbiota Commensal bacteria can induce iNOS ⁺ TNF ⁺ DC that promote IgA responses Commensal bacteria induce regulatory cytokine production by DC, such as IL-10 and TGFβ, and also regulatory mediator RA	Direct modulation by microbiota CX3CR1 ⁺ MΦ directly sample luminal antigens; this process is dependent on the microbiota	Indirect modulation by microbiota DC and MΦ sampling commensal bacteria induce IgA production by B-cells <i>via</i> BAFF and APRIL release, and production of IgA-inducing cytokines IL-10 and TGFβ

TGFβ: Transforming growth factor beta; TNF: Tumor necrosis factor; DC: Dendritic cells; MΦ: Macrophages; IL-10: Interleukin 10; T-reg: Regulatory T cells; TLR: Toll-like receptors; pDC: Plasmacytoid DC; RA: Retinoic acid; HLA: Human leukocyte antigen; IBD: Inflammatory bowel disease.

gration and survival. However, this process is dependent on the presence of exogenous RA^[141].

Regulatory effects of DC/B-cell interactions are not restricted to induction of IgA production by B-cells; B-cell conversion into immunosuppressive B-cells (regulatory B-cells) is partially dependent on DC production of RA^[142]. However the *in vivo* effects of intestinal DC and MΦ on conversion of gut-specific regulatory B-cells is unknown. Crosstalk between intestinal DC and B-cells can also lead to active immunity as well as regulatory immune responses; murine CD11b⁺ CD11c⁺ DC from the small intestine lamina propria express TLR5 and respond to flagellin from flagellated bacteria by inducing IgA⁺ plasma cells and antigen-specific Th17 and Th1 subsets to generate protective immunity^[138]. Crosstalk between gut DC/MΦ is bidirectional; immunoglobulins secreted by B-cells can have a direct effects on DC differentiation and activation^[143,144], though this process at intestinal sites has not been described in detail.

Role of the gut microbiota in antigen-presenting cell crosstalk

In response to pathogenic bacteria or the commensal

microbiota, APC contribute to both innate and adaptive immune responses that can be either immunogenic or tolerogenic through TLR stimulation^[11,145]. TLR-dependent activation of DC in particular can determine protection or immune tolerance that maintains immune homeostasis at intestinal sites^[146,147]. Commensal bacteria can induce intestinal iNOS⁺/TNF⁺ DC that, in the intestinal lamina propria, promote IgA responses by releasing BAFF and APRIL in response to nitric oxide^[148]. These DC may also enhance IgA responses in Peyer's patches by upregulating expression of TGFβ receptor type II on follicular B-cells^[148]. IgA-inducing cytokines including IL-10 and TGFβ are secreted by DC and MΦ in response to microbial TLR ligands^[118,156]. Gut DC also produce RA and IL-6 in response to microbial TLR ligands^[94,136] which have direct effects on both B-cell secretion of IgA and imprinting gut-homing markers on B-cells^[94], as mentioned above.

Non-migratory APC expressing CX3CR1, likely to be MΦ^[15], continuously sample antigens by extending transepithelial projections without disrupting tight junctions^[149]; CX3CR1⁺ APC may be able to directly present commensal antigens to subepithelial B-cells^[43,47]; Murine

intestinal APC initiate production of commensal reactive IgA by presenting commensal bacterial antigens to B-cells^[9,150]. It is likely that CX3CR1⁺ APC transfer antigen to CD103⁺ migratory DC prior to DC migration to secondary lymphoid tissue to prime tolerogenic T-cell responses towards the microbiota^[15]. Commensal bacteria play a critical role in antigen sampling by CX3CR1⁺ APC as antibiotic treatment reduces the number of transepithelial projections^[145,151]. However, it has recently been shown that CX3CR1^{hi} cells can migrate to MLN and traffic *Salmonella* antigen to induce T-cell responses and IgA production in the absence of MyD88 or following antibiotic treatment^[65]. In this study, MyD88-dependent recognition of commensal bacteria in the gut reduced bacterial trafficking to MLN by CX3CR1^{hi} APC to down regulate excess inflammation and autoimmunity.

A subset of intestinal pDC expressing TLR7 and TLR9 are capable of producing high levels of type I IFN^[152] which in turn promotes not only maturation and differentiation of myeloid DC, but also class-switching of antibodies produced by B-cells^[153] (Table 1).

CONCLUSION

DC, MΦ and B-cells are professional APC that are fundamental components of both the innate and adaptive immune system in the gut; their plasticity allows these cells to function in an environment where they are constantly exposed to the commensal microflora and food antigens, but can also be exposed to harmful pathogens. Intestinal APC are individually specialized to perform specific functions but their role in shaping both primary and secondary T-cell responses, including generation and differentiation of T-regs, highlights their importance in intestinal immune homeostasis and IBD pathogenesis. APC function in the gut is in turn directly shaped by the microbiota. Furthermore, crosstalk between APC is essential for intestinal immunity and tolerance. Clarification and a better understanding of the functions of intestinal APC subsets, especially in humans, may provide novel therapeutic targets for manipulating mucosal immunity and tolerance, leading to new and more effective treatment for IBD.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Relationship between pouch microbiota and pouchitis following restorative proctocolectomy for ulcerative colitis

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Abstract

Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) has become the surgical treatment of choice for many patients with medically refractory ulcerative colitis (UC) and familial adenomatous polyposis (FAP). UC patients with IPAA (UC-IPAA) are, nevertheless, susceptible to inflammatory and noninflammatory sequelae such as pouchitis, which is only rarely noted in FAP patients with IPAA. Pouchitis is the most frequent long-term complication of UC-IPAA patients, with a cumulative prevalence of up to 50%. Although the aetiology of pouchitis remains unclear, accumulating evidence suggests that a dysbiosis of the pouch microbiota and an abnormal mucosal immune response are implicated in its pathogenesis. Studies using culture and molecular techniques have detected a dysbiosis of the pouch microbiota in patients with pouchitis. Risk factors, genetic associations, and serological markers suggest that interactions between the host immune response and the pouch microbiota underlie the aetiology of this idiopathic inflammatory condition. This systematic review focuses on the dysbiosis of the microbiota

that inhabit the pouch in UC and FAP patients and its interaction with the mucosal immune system. A meta-analysis was not attempted due to the highly heterogeneous microbiota composition and the different detection methods used by the various studies. Although no specific bacterial species, genus, or family has as yet been identified as pathogenic, there is evidence that a dysbiosis characterized by decreased gut microbiota diversity in UC-IPAA patients may, in genetically predisposed subjects, lead to aberrant mucosal immune regulation triggering an inflammatory process.

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Key words: Pouchitis; Inflammation of the ileal pouch; Microbiota; Bacteria; Microbiome; Ileal-pouch-anal anastomosis; Ulcerative colitis; Crohn's disease; Inflammatory bowel disease

Core tip: This is a systemic review assessing the relationship between the microbiota that inhabit the ileal-anal pouch following restorative proctocolectomy in ulcerative colitis and familial adenomatous polyposis patients and the inflammatory response that can occur. A meta-analysis was not attempted in view of the highly heterogeneous microbiota composition and the different detection methods utilized. Although no specific bacterial species, genus, or family has as yet been identified as pathogenic, there is evidence that dysbiosis and reduced bacterial diversity of the microbiota found in ulcerative colitis patients who have undergone restorative proctocolectomy may, in genetically predisposed subjects, lead to aberrant mucosal immune regulation triggering an inflammatory process.

Original sources: Angriman I, Scarpa M, Castagliuolo I. Relationship between pouch microbiota and pouchitis following restorative proctocolectomy for ulcerative colitis. *World J Gastroenterol* 2014; 20(29): 9665-9674 Available from: URL:

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INTRODUCTION

About 20%-25% of patients with ulcerative colitis (UC) require a colectomy at some point in their lives, and in most cases the operative surgical procedure chosen is a restorative proctocolectomy (RPC) with ileal pouch-anal anastomosis (IPAA)^[1,2]. Diseased colonic/rectal tissue is removed during the procedure, and transanal fecal continence is maintained by creating an ileal pouch. Although effective, inflammation of the ileal pouch (pouchitis) is a common complication, with almost 50% of patients experiencing an acute episode within 5 years and about 5% of those going on to develop chronic inflammation^[1-5]. The cumulative incidence of pouchitis in patients who undergo an IPAA for familial adenomatous polyposis (FAP) is much lower, ranging from 0% to 10%^[6]. Reasons for the higher frequency of pouchitis in UC remain unknown.

Common clinical symptoms in patients with pouchitis include increased bowel movements, abdominal pain/cramping, urgency, incontinence, generalized fatigue/malaise, fever, and, occasionally, bloody stools. The diagnosis of pouchitis is based on assorted clinical, endoscopic, and histologic findings, including endoscopic and microscopic evidence of inflammation of the ileal pouch^[7]. In the absence of evident signs of inflammation of the terminal ileum, fecal lactoferrin, or calprotectin (mucosal inflammation markers) can help to distinguish pouchitis from irritable pouch syndrome^[8]. Once diagnosed, disease activity can be quantified using the pouchitis disease activity index (PDAI), which takes into consideration clinical findings, histology, and laboratory parameters^[9].

Histological features of pouchitis can also be non-specific, including acute inflammation with polymorphonuclear leukocyte infiltration, crypt abscesses, and ulceration in association with a chronic inflammatory infiltrate^[7,10]. A discrepancy between endoscopic and histologic findings, possibly linked to sampling errors, has been noted in patients with pouchitis^[11,12]. Morphological alterations of the epithelium lining the ileal pouch, characterized by flattening and a reduced number or complete disappearance of the villi leading to villous atrophy (colonic metaplasia), normally develop within 12-18 mo after ileostomy closure^[10,12]. Although a causal association has not been proven, once colonic metaplasia has developed in the pouch, pouchitis may occur^[13].

In accordance with Mahadevan and Sandborn's definition, the pattern of pouchitis is classified as infrequent (1 or 2 acute episodes), relapsing (3 acute episodes), or continuous. Recurrent pouchitis is defined, according to this classification, as relapsing (more than two episodes) or chronic^[13]. Chronic pouchitis is usually refractory to medical therapy and/or requires maintenance therapy, and may lead to pouch excision and permanent terminal

ileostomy^[13]. Risk factors linked to pouchitis include extensive UC^[2,14], backwash ileitis^[14], extraintestinal manifestations, chiefly primary sclerosing cholangitis^[15-17], being a non-smoker, and regular use of nonsteroidal anti-inflammatory drugs^[18,19].

Genetic polymorphisms in interleukin-1 receptor antagonist (IL-1Ra) or the presence of perinuclear neutrophil cytoplasmic antibodies^[20] have also been associated with pouchitis. Although definitive proof is still lacking^[21], indirect evidence from clinical practice supports the hypothesis that the pouch microbiota (*i.e.*, a microbial imbalance) plays an important role in the pathogenesis of pouchitis^[22,23]. It has been seen, in fact, that mucosal inflammation is localized in the area of the gut characterized by the highest bacterial concentrations^[24] and is limited to the mucosal surface^[25]. Short-term, oral antibiotic therapy (*i.e.*, metronidazole or ciprofloxacin)^[4,26] has been reported to be an effective treatment for both pouchitis and pre-pouch ileitis in up to 87.5% of patients^[27,28]. Probiotics have also been shown to reduce disease relapse and the risk of disease onset^[29]. Findings on risk factors, genetic associations, and the serological markers of pouchitis all seem to point to the conclusion that host immune responses and pouch microbiota interactions trigger this idiopathic inflammatory condition^[30].

SEARCH STRATEGY

Inclusion and exclusion criteria

Studies focusing on the analysis of the gut microbiota following IPAA in patients with UC or FAP were eligible for inclusion. Studies examining pouch disease were also included, with microbiota analysis being carried out in at least 10 patients. Studies or case reports dealing exclusively with operative or postoperative management or referring to fewer than 10 patients following IPAA were excluded. Studies reporting on partial analysis of the microbiota or on patients with anastomosis for Crohn's disease (CD) were also excluded. Only studies providing information on analysis of the entire bacterial microbiota were included. Whenever publications reporting on overlapping patient data were being considered, only the most complete and recent set of data were included.

Search strategy

With the assistance of a clinical librarian, two researchers (Angriman I and Scarpa M) consulted Medline, the Embase Medical Database, the Cochrane Database of Systematic Reviews, and the Cochrane Central Register of Controlled Trials (CENTRAL) for studies concerning ileal pouches carried out between January 1978 (publication date of the first manuscripts on RPC) and October 2013. The keywords and Medical Subject Headings (MeSH) used were "pouchitis OR chronic pouchitis OR acute pouchitis OR inflammation of the ileal pouch AND microbiota OR bacteria OR microbiome". Only clinical studies in English, Dutch, Spanish, German, French, and/or Italian were considered. A manual cross-reference

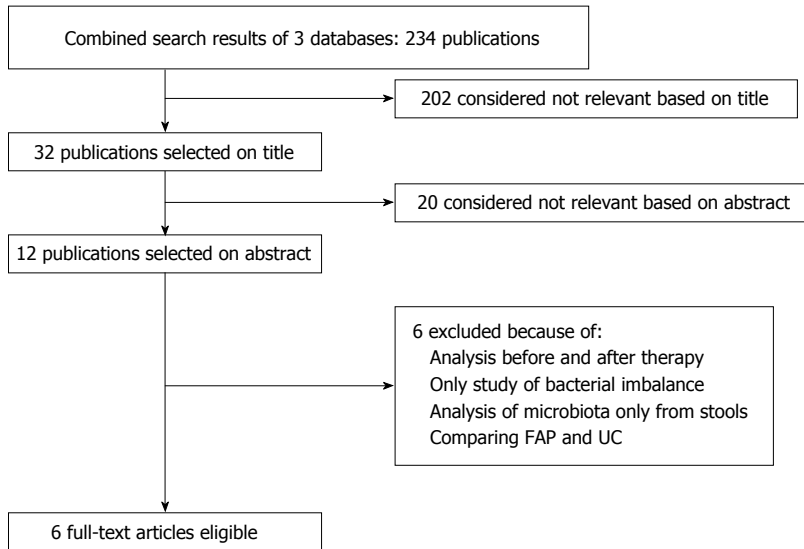


Figure 1 Flow diagram of literature review, eligibility determination, and inclusion in the systematic review and meta-analysis. FAP: Familial adenomatous polyposis; UC: Ulcerative colitis.

search for qualified papers was also carried out to identify additional relevant articles, and the resulting studies that were compatible with the inclusion criteria were evaluated independently. Unpublished data or findings published in abstracts were not taken into consideration. A negotiated agreement was reached between the two researchers whenever there was discordance regarding study inclusion.

Data extraction

Only data from original articles were extracted using a preformatted sheet with spaces for the following parameters: demographic data, pouchitis symptoms, presence of GI bleeding, frequency of bowel movements, abdominal pain, PDAI score, and endoscopic and histological diagnoses (Figure 1).

STATISTICS

Review Manager 4.2 software (The Cochrane Collaboration, Copenhagen: The Nordic Cochrane Centre, 2003) was used for all statistical analyses. A meta-analysis was not attempted due to the highly heterogeneous microbiota composition and the different detection methods used.

POUCH BACTERIOLOGY AND POUCHITIS

The composition of the bacterial microbiota in the terminal ileum differs significantly from that found in the colon^[31,32], and there are likewise significant differences in the microbial composition of the luminal and mucosal compartments of the gut^[33]. Moreover, it is known that the gut microbiota profoundly influences the intestinal mucosa and gut-associated lymphoid tissue^[34]. Although the microbiota that colonizes the mucosal surface usually exercises beneficial trophic, immunomodulatory, and anti-inflammatory effects, an “imbalanced” microbiota can damage the mucosa by producing toxins or triggering ab-

normal immune responses^[35]. Alterations in the intestinal mucosal-associated microbiota have, in fact, been linked to the pathogenesis of several gastrointestinal inflammatory disorders.

Many of the studies concerning the microbiota after IPAA that were carried out prior to the formulation of PDAI in 1994 show inconsistencies in their definition and diagnosis criteria, as well as in the distinction between acute and chronic pouchitis^[9]. In addition, the majority of studies focusing on the pouch microbiota also used culture methods, despite 60%-80% of gut bacterial species being unculturable^[36,37]. Variability in the use of fecal or mucosal samples further contributed to the discrepancies in findings noted in many of these studies.

It is well-established that, within the first year after ileostomy closure, the overall composition of microbiota shows similarities with that of the colon^[38,39]. A number of studies using fecal cultures to evaluate the microbiota of pouches in UC and FAP patients^[40,41] produced conflicting results with regard to the ratio of anaerobic bacteria to aerobic bacteria, total bacterial counts, and sulfate-reducing bacteria^[42] in the pouchitis and in non-pouchitis patients. The high grade of variability in these kinds of studies may be due to the daily variability of stool composition in relation to diet. Moreover, the high frequency of bowel movement in IPAA patients may enhance this variability.

The mucosal-adherent microbiota, which is in close contact with the gut mucosa, has recently been shown^[37,43] to be distinct from the luminal and fecal ones, which are made up of free-living or particle-attached cells. The differences in community structure are probably linked to a number of factors, such as differential substrate availability (mucus *vs* undigested dietary residues), oxygen levels, and host-microbe interactions. In particular, mucosa adherent microbiota may be influenced by drugs. The close proximity of the mucosal-adherent microbiota to the

gut epithelium suggests that these bacteria may be more relevant than the luminal microbiota in the pathogenesis of inflammatory bowel disease (IBD), since they, as well as their excreted products, probably have direct contact with the host^[37]. Moreover, since they live in a mucous environment their populations are more protect, and thus more constant.

The ideal microbiota analysis probably would examine both the fecal and mucosal-adherent microbiota. In fact, fecal microbiota may give a rough but more complete idea of whole bowel microbiota, while mucosa adherent bacteria directly cross-talk with the host and are more likely to be involved in the pathogenesis of pouchitis.

Knowledge about the complexity and diversity of the gut microbiota underwent a radical revision when 16S rRNA techniques were introduced^[31,32], with 16S rRNA gene sequencing results representing gene copy number rather than true bacterial counts. Possibly biased by differential DNA extraction and PCR amplification rates, the methodology represents, nevertheless, the best available option, and is considered the “gold standard” for the analysis of gut-associated complex microbial communities^[44]. Some studies used molecular techniques to show that microbiota that inhabit the ileal-anal pouch differ from that of the normal large intestine. UC pouches with or without pouchitis appear to harbor particularly more unusual microbes. Proteobacteria, which normally account for only a small proportion of the microbiota in the healthy colon^[31] and were found to make up 20% in IBD patients^[32], comprised up to 90% (median = 66.6%) in UC-IPAA patients in one study^[45]. That same study also showed lower than normal proportions of *Bacteroidetes*, *Lachnospiraceae* and *Ruminococcaceae*. A comparison of the two study cohorts revealed that the UC-IPAA patients had increased proportions of the phylum *Proteobacteria* and decreased levels of *Bacteroidetes* with respect to the FAP-IPAA patients.

Consistent with a reduced bacterial diversity observed in both CD^[46] and UC^[47,48], another study demonstrated that there is a significantly lower bacterial diversity in UC-IPAA patients, with or without pouchitis, with respect to FAP-IPAA patients^[49]. However, only minor compositional differences were detected in the microbiota of UC patients with active pouchitis with respect to those with no disease history. The authors of that study hypothesized that dysbiosis predisposes UC patients to pouchitis either by increasing the likelihood of immune system stimulation or by reducing microbiota diversity, which may itself be sufficient to stimulate the immune system, leading to mucosal inflammation. The observation that VSL#3 administration increases bacterial diversity and thus reduces relapse risk^[50] supports the second hypothesis. Zella *et al.*^[45] demonstrated that the microbial environment in the pouches of UC-associated, healthy UC and FAP patients is distinctly different. Using 16S ribosomal gene-based Terminal Restriction Fragment Length Polymorphism (TRFLP) data, those investigators identified significant differences in fecal and mucosal

bacterial communities in the three patient cohorts. Broad differences in TRFLP profiles were further analyzed using DNA sequencing, which revealed multiple significant variations in specific bacterial genera in the pooled fecal bacterial DNA samples in the UC and FAP groups.

Tannock *et al.*^[51] demonstrated that bacteria uncommonly present in the stools of humans in general and in FAP patients in particular comprised about 50% of the microbiota of patients with pouchitis when antibiotic-treated (CP-off = asymptomatic), and that antibiotic treatment reduced the proportion of the unknown bacteria in their feces. Chronic or recurrent pouchitis was therefore found to be associated with microbiota containing bacteria not commonly associated with human feces or FAP pouches. The uncommon bacteria constituting a large proportion of the CP-off but not of the untreated CP-on (symptomatic) microbiota were found to be members of the *Caulobacteraceae*, *Sphingomonadaceae*, *Comamonadaceae*, *Peptostreptococcaceae* and *Clostridiaceae*. At least some of these groups could theoretically be linked to the pathogenesis of pouchitis. The investigators also noted a wide diversity in the clostridial operational taxonomic units in the CP-off microbiota, presumably denoting particularly favorable conditions in the pouch for the expansion of clostridial populations. The authors concluded that *C. perfringens* may play a role in the etiology of pouchitis in some patients.

Although it is not clear if reduced biodiversity causes, perpetuates, or is a result of IBD, other studies have also demonstrated a quantitative and qualitative (biodiversity) reduction in the representation of the Firmicutes phylum, particularly clostridial cluster IV members, in the feces of CD patients. This phylogenetic group contains several butyrate-producing bacteria, such as *Faecalibacterium prausnitzii* (*F. prausnitzii*). Butyrate and other short chain fatty acids are considered important energy sources for colonic epithelial cells, have anti-inflammatory properties, and improve the intestinal barrier function of epithelial cells. A reduction in butyrate-producing bacteria in the colon or pouch might then have an overall detrimental effect on the gut mucosa^[52-56]. Indeed, although *F. prausnitzii* was not detected in chronic pouchitis stool microbiota, low levels were found in FAP and normal pouch feces. Members of the *Lachnospiraceae*, some of which produce butyric acid, were depleted in chronic pouchitis microbiota in FAP and normal pouches (average 33.61% and 21.86%, respectively). The authors reported that normal and FAP pouches showed similar proportions of *Lachnospiraceae* and clostridial cluster IV, indicating that measures of these bacterial groups in the microbiota could be useful biomarkers of pouch health.

In a recent study, we reported that the *Enterobacteriaceae*, *Streptococcaceae*, *Enterococcaceae*, and *Bacteroidaceae* were the most frequent strains of cultivable bacteria adherent to the pouch mucosa, while *Lachnospiraceae*, *Fusobacteriaceae*, *Veillonella*, *Staphylococcaceae*, *Bifidobacteriaceae*, *Eubacteriaceae*, *Bacillaceae*, *Moraxellaceae*, *Burkholderiaceae* and *Corynebacteriaceae* were the least frequent ones. Although

their incidence in the pouch mucosa was similar in the chronic pouchitis and normal pouch groups, we detected a significantly higher incidence of *Clostridiaceae spp.* in the chronic/recurrent pouchitis group compared to that of the normal pouch. The presence of *Clostridiaceae spp.* was found to be an independent predictor of chronic/relapsing pouchitis^[57] at multiple logistic regression analysis. In another study, we found that patients with a clinical diagnosis of pouchitis had a significantly reduced level of *Enterococcaceae spp.* (including bacteria strictly adherent to the pouch mucosa and in the pouch mucous) *vs* those without a clinical diagnosis. Total *Enterobacteriaceae spp.* and *Streptococcaceae spp.* counts in the mucosa were also decreased in patients with clinical pouchitis compared with patients with “healthy” pouches. Our findings indicate that *Bacteroidaceae spp.* and *Clostridiaceae spp.* are more frequently associated with microscopic inflammation of the pouch mucosa and may therefore play a pathogenic role in pouchitis. *Enterococcaceae spp.*, as well as possibly *Enterobacteriaceae spp.* and *Streptococcaceae spp.*, may instead play an active role in maintaining immunologic homeostasis within the pouch mucosa, with low levels feasibly favoring the development of pouchitis^[58].

Zella *et al.*^[45] similarly noted an overall increase in fecal *Clostridium spp.* in UC-associated pouchitis with respect to that in FAP patients. They also noted a reduction in *Bacteroides spp.* in the inflamed pouch with respect to FAP pouches, confirming that a mucosal and luminal dysbiosis exists in pouchitis not only when compared to the healthy UC pouch but also to a non-IBD one.

The overall decrease in the Bacteroidetes phylum in the inflamed pouch is consistent with several studies that included data on the molecular analysis of intestinal microbiota in both CD and UC^[47,59,60]. In line with previous reports, those studies also found that healthy UC pouches differed significantly from FAP ones, leading to the hypothesis that an alteration in the ileal pouch microbiota may be exclusive to the UC disease state, with or without inflammation. In particular, since Bacteroidetes may play a key role in maintaining gut health, a relative reduction in this population may favor inflammation^[45]. In addition, those authors found a significant increase in clostridia, namely among the *Clostridium*, *Lachnospiraceae*, and *Roseburia* genera, in the inflamed pouch. *Roseburia* are flagellated commensal inhabitants of the colon. Flagellin has been shown to induce proinflammatory gene expression by activating toll-like receptor 5 (TLR5); subjects with TLR5 polymorphisms and low levels of anti-flagellin antibodies may be protected from developing CD. Present in 50% of CD and 6% of UC patients, high levels of anti-flagellin antibodies, specifically anti-CBir1 antibodies to flagellin of *Clostridium spp.*, may be associated with the development of pouchitis^[61,62]. Additionally, the mucin-degrading *Akkermansia* genus of the Verrucomicrobia phylum was significantly more prevalent in pouchitis. There are several theories regarding the role of mucin in protecting the intestinal epithelium in IBD, with mutations, alterations, and degradation of mucins being

associated with CD and UC^[63-66].

In conclusion, studies using molecular techniques to analyze the microbiota have confirmed that there is dysbiosis of the pouches of UC-IPAA patients; there are, however, conflicting results with regard to differences in the abundance of particular species and phylotypes associated with pouchitis and in the degree of community diversity of the pouch microbiota (Table 1). As dysbiosis of the gut microbiota could be a pathogenic prerequisite for the idiopathic development of gut inflammation, this would explain the higher frequency of pouchitis in UC-IPAA with respect to FAP-IPAA patients. Although dysbiosis is found in the UC pouch, it may not be the single cause leading to inflammation, but rather a predisposing factor.

POUCH IMMUNE RESPONSES TO BACTERIA

While some investigations have failed to identify a specific microbe as a causative agent^[23], others have stated that they believe sulfate-reducing bacteria, such as *Clostridiaceae spp.*, *Enterobacteriaceae spp.* and *Bacteroidaceae spp.*, might be implicated in the pathogenesis of pouchitis^[67-71]. Our group recently observed that *Clostridiaceae spp.* adherent to pouch mucosa are associated with chronic/relapsing pouchitis while *Enterococcaceae spp.* and possibly *Enterobacteriaceae spp.* and *Streptococcaceae spp.* may play a role in maintaining immunologic homeostasis within the pouch mucosa, with low levels of these bacteria favoring the development of acute pouchitis^[57,58]. Deregulated mucosal cell immunity toward the microbiota also seems to be implicated in the pathogenesis of pouchitis. Epithelial and immune cells within the intestinal mucosa recognize conserved microbial structures, known as pathogen-associated molecular patterns (PAMPs), through membrane-bound and cytoplasmic receptors [so-called pattern recognition receptors (PRRs)], which, when activated, trigger intracellular signaling pathways to elicit a variety of stereotyped (so-called innate) immune responses^[72].

Fragments of bacterial peptidoglycan (the main component of the bacterial cell wall) can, for example, serve as PAMPs that bind to the PRR toll-like receptor 2 (TLR2), while bacterial lipopolysaccharides (LPS; found in the outer membrane of gram-negative bacteria) can serve as PAMPs that bind to TLR4. Binding of these conserved bacterial structures to TLRs triggers a number of intracellular signaling cascades which, through NF- κ B and protein kinase activation, lead to the transcription of a series of genes^[73]. In macrophages this activation results in the expression of several pro-inflammatory cytokines [including interleukin (IL)-1 β , tumor-necrosis factor (TNF)- α , and IL-6], chemokines, adhesion molecules, leukotrienes, nitric oxide, and production of reactive oxygen species, while in dendritic cells it enhances major histocompatibility complex class II and co-stimulatory molecule expression favoring subsequent antigen-specific, T-helper cell activation^[74,75]. Microbiota associated

Table 1 Studies on the pouch microbiota in which molecular methods were used

Ref.	Year	Microbiota identification	Sampling and analysis	Normal pouch (n)	Pouchitis (n)	Bacteria normal pouch	Bacteria pouchitis	Taxonomy level	Methods	Database
Komanduri <i>et al.</i> ^[69]	2007	16S rRNA	Mucosa adherent microbiota	15	5	<i>Clostridium parvutrificum</i> <i>E. coli/Shigella</i> spp.	<i>Fusobacterium varium</i>	Genus and species	LH-PCR	PCO plot
Lim <i>et al.</i> ^[21]	2009	Technique (LH-PCR)								
McLaughlin <i>et al.</i> ^[69]	2010	16S rDNA PCR products	Stool microbiota	15	5	No differences	<i>Ruminococcus obeum</i>	Genus and species	T-RFLP	BLAST
		16S rDNA PCR products	Mucosal adherent microbiota	8	8	No differences		Phylum, family and species	PCR	Mega BLAST
Zella <i>et al.</i> ^[45]	2011	16S rRNA PCR products	Stool microbiota	3	9	Bacteroidetes (<i>vs</i> FAP)	<i>Clostridium</i> <i>Eubacterium</i> <i>Firmicutes</i> (<i>vs</i> FAP)	Phylum, Family Genus, species	RFLP	BLAST
Scarpa <i>et al.</i> ^[38]	2011	16S rDNA PCR products	Mucosa adherent microbiota culture	22	10	<i>Enterobacteriaceae</i> <i>Enterococcaceae</i>	<i>Clostridiaceae</i> <i>Bacteroidaceae</i>	Family	GAPDH, qRT-PCR	BLAST
Tyler <i>et al.</i> ^[60]	2013	16S rDNA PCR products	Mucosa adherent microbiota	19	15	<i>Bacteroides</i> <i>Sutterella</i> <i>Blautia</i> <i>Moryella</i> <i>Dorea</i> <i>Pantbacteroides</i>	<i>Proteobacteria</i> <i>Firmicutes</i> <i>Roseburia</i> spp. <i>Eubacterium rectale</i>	Phylum, genus	qPCR	Mothur

FAP: Familial adenomatous polyposis. *E. coli*: *Escherichia coli*; LH-PCR: Length Heterogeneity Polymerase Chain Reaction.

PAMPs modulating the release of a number of biologically active molecules profoundly influence the function and integrity of the intestinal mucosa.

Experimental findings indicate that these mechanisms are activated in pouchitis. In a recent study, Toiyama *et al.*^[70] reported that TLR-2 and TLR-4 were greatly up-regulated in the mucosa of active pouchitis, while TLR-3 and TLR-5 expression was barely detectable in the normal ileum and not detectable at all in pouch mucosa with or without inflammation. We confirmed in a recent study that high mucosal TLR-2 and TLR4 mRNA levels are associated with chronic/relapsing pouchitis^[57].

Epithelial cells are also equipped with various antimicrobial peptides that act rapidly to kill a wide range of microorganisms. Defensins are an important class of antimicrobial peptides, which are small cationic arginine-rich peptides with a molecular weight of 3-5 kDa expressed exclusively in Paneth cells (α -defensins) or generally in intestinal epithelial cells (β -defensins)^[74,75]. They are classified as α - and β -defensins depending on the position of three intramolecular disulfide bridges between cysteine residues. The α -defensins include the human neutrophil peptides 1-4, as well as epithelial human defensin 5 (HD-5) and human defensin 6 (HD-6). α -defensins, which may act as chemokines, have a wide variety of antimicrobial activities^[77-79]. HD-5 and HD-6 are the major antimicrobial peptides of the small intestine, and their expression is increased in the colonic mucosa of IBD patients in the form of metaplastic Paneth cells^[80,83]. In contrast with enteric α -defensins, human β -defensin 1 (HBD1) and other members of the β -defensin family appear to be expressed by most epithelial cells of the small and large intestines. HBD-1 is expressed constitutively, while HBD-2 and HBD-3 expression require NF- κ B activation^[84]. Microbes or their products could be involved in defensin deregulation in normal pouches after surgery and in the presence of pouchitis^[85]. A recent study reported that defensins are up-regulated in response to bacterial PAMPs or cytokines^[86]. The increase in HBD-2 and HBD-3 in pouchitis may thus reflect mucosal damage and infection^[85]. The interplay between the microbiota adherent to the pouch mucosa, the mucosal innate immune system, and defensin expression may thus contribute to the pathogenesis of pouchitis.

Some evidence suggests that there is a disruption in the host-gut microbiota homeostasis^[67-71] associated to (or even caused by) the deregulation of the innate immunity machinery within the ileal mucosa^[73,76]. In fact, in recent studies, we observed that *Enterococcaceae* spp., *Enterobacteriaceae* spp. and *Streptococcaceae* spp. may play an active role in maintaining immunologic homeostasis within the pouch mucosa, as low levels seem to favor the development of acute pouchitis^[21]. The presence of *Clostridiaceae* spp. adherent to the pouch mucosa and the high TLR-2 and TLR-4 mRNA expressions were, moreover, associated with chronic/relapsing pouchitis^[57].

CONCLUSION

Accumulating experimental evidence indicates that dysbiosis of the ileal-anal pouch microbiota and deregulation of the mucosal immune system play an important role in the pathogenesis of pouchitis. While its diversity was reduced, only minor compositional differences were found in the microbiota of UC patients with active pouchitis with respect to that in subjects without disease history. However, there are no studies in the literature which have analyzed the direct impact of pouchitis therapy on pouch microbiota. The most likely hypothesis is that dysbiosis predisposes UC patients to pouchitis by increasing the likelihood of immune system stimulation or by reducing microbiota diversity, which is itself sufficient to induce unbalanced activity of the immune system leading to mucosal inflammation. The failure to identify a particular bacterial species associated with pouchitis concurs with clinical experience indicating that antibiotics with a very different spectrum of antimicrobial activity are equally effective in pouchitis. Some investigators have recently shown that many patients with the form that is refractory to empirical antibiotic treatment have antibiotic resistant coliforms, and microbiological testing has been able to predict an effective antibiotic regime for those patients^[87]. Together with other findings, this suggests that antibiotic therapy is effective in pouchitis as it reduces the total gut microbial load, and therefore the stimulus to the immune system rather than eliminating a specific disease-activating bacterial species. Although the connection between dysbiosis of the microbiota and aberrant immune responses remains unclear, alterations in the mucosal immune system support the hypothesis that dysbiosis plays an active role in inducing and maintaining persistent inflammation. The dysbiosis in UC-IPAA patients, characterized by reduced diversity of the microbiota, may lead to aberrant mucosal immune regulation triggering the inflammatory processes in genetically predisposed patients. Secondary effects on the function of the epithelial membrane barrier and defensin overexpression could subsequently worsen dysbiosis and favor the chronic activation of mucosal immune responses^[88].

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Optimization of the treatment with immunosuppressants and biologics in inflammatory bowel disease

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Abstract

Many placebo controlled trials and meta-analyses evaluated the efficacy of different drugs for the treatment of inflammatory bowel disease (IBD), including immunosuppressants and biologics. Their use is indicated in moderate to severe disease in non responders to corticosteroids and in steroid-dependent patients, as induction and maintenance treatment. Infliximab, as well as cyclosporine, is considered a second line therapy in the case of severe ulcerative colitis, or non-responders to intravenous corticosteroids. An adequate dosage and duration of therapy with thiopurines should be reached before evaluating their efficacy. Methotrexate is a valid option in patients with Crohn's disease but its use is confined to patients who are intolerant or non-responders to thiopurines. Evidence for the use of methotrexate in ulcerative colitis is insufficient. The use of thalidomide and mycophenolate mofetil is not recommended in patients with inflammatory bowel disease, these treatments could be considered in case of failure of all other therapeutic options. In patients with moderately active ulcerative colitis, refractory to thiopurines, the use of tacrolimus is considered an alternative to biologics. An increase of the dose or a decrease in the interval of administration of biological treatment could be useful in the presence of an incomplete clinical response. In the case of primary failure of an anti-tumor necrosis factor

alpha a switch to another one should be considered. Data on the efficacy of combination therapy are up to now insufficient to consider this strategy in all IBD patients. The final outcome of the treatment should be considered the clinical remission, with mucosa healing, and not the clinical response. The evaluation of serum concentration of thiopurine methyl transferase activity, thiopurine metabolites, biologic serum levels and anti-biologic antibodies could be useful for the management of the treatment but it has not been routinely applied in clinical practice. The evidence of high risk development of lymphoma and cutaneous malignancies should be considered in patients treated with immunosuppressants and biologics for a long period.

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Key words: Inflammatory bowel disease; Optimization; Immunosuppressants; Biologics; Crohn's disease; Ulcerative colitis

Core tip: The clinical expression of inflammatory bowel disease (IBD) is heterogeneous with different clinical courses, so it is not easy to find the best therapy for all patients. In recent years the goals of the therapy for IBD patients have evolved from symptomatic control to altering the course of disease by achieving a "deep remission". Many trials have evaluated the efficacy of immunosuppressants and biologics in achieving clinical and endoscopic remission but the optimization of these treatments is still a debated point. We propose some recommendations about the correct use of immunosuppressants and biologics for the treatment of IBD, based on the current evidence.

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INTRODUCTION

Inflammatory bowel disease (IBD) refers to 2 chronic inflammatory disorders of the gastrointestinal tract: Crohn's disease (CD) and ulcerative colitis (UC). The clinical expression of IBD is heterogeneous, especially in CD, with a wide spectrum of patterns and different clinical courses, so it is not easy to find the best therapy for all patients. The course of IBD is characterized by phases of relapse and remission and the main goal of the therapy is to achieve and maintain disease remission. In recent years the goals of therapy for IBD patients have evolved from symptomatic control to altering the course of disease by achieving a "deep remission". This is defined as the contemporary presence of sustained clinical remission, complete mucosal healing and normalization of serological activity indexes (C-reactive protein and erythrocyte sedimentation rate). Many trials have evaluated the efficacy of different drugs for the treatment of CD and UC, including immunosuppressants and biologics. Although recent guidelines have been published to direct the clinicians on the correct management of IBD^[1-3], up to now the optimization of treatment with immunosuppressants and biologics is a debated point. However, nearly half of the IBD guideline recommendations are based on expert opinion and the guidelines are not frequently updated^[4]. We propose some recommendations about the correct use of immunosuppressants and biologics for the treatment of CD and UC, based on the current evidence.

CROHN'S DISEASE

Immunosuppressant therapies

Although the efficacy of corticosteroids in active CD is clear, their use is not indicated in maintaining treatment and the long-term therapy is associated with serious adverse consequences^[5]. To avoid the long term use of corticosteroids many studies have investigated the efficacy of immunosuppressants, including thiopurines, methotrexate (MTX), tacrolimus, thalidomide and mycophenolate mofetil (MMF) for the treatment of active and quiescent CD.

Thiopurines

6-mercaptopurine (6-MP) and its prodrug azathioprine (AZA) are purine analogues able to reduce cell proliferation and with immune modifier properties. The efficacy of thiopurines for the treatment of active CD is controversial. In a meta-analysis^[6] evaluating 8 trials on AZA and 6-MP therapy in patients with active CD, a higher response rate was observed in patients treated with thiopurines compared with patients treated with placebo. The time of peak response in the included trials ranged from 9 wk to more than 26 wk; the minimum period for an adequate response was found to be more than 17 wk. A more recent meta-analysis^[7], including 5 trials, showed no significant efficacy of thiopurines in active CD. The discrepancy between the results of the two meta-analyses is probably due to the exclusion, in

the second one, of trials evaluating the outcome after 17 wk of follow up. This discrepancy confirms that a longer time of treatment gives a greater chance of obtaining a positive result. Based on these data the use of thiopurines as a single therapy is not recommended in active CD but a combination with corticosteroids could be useful while waiting for the slow time of action of thiopurines. Unfortunately treatment with thiopurines is sometimes associated with the occurrence of side effects that preclude their use. In these cases the discontinuation of treatment causes a complete resolution of the symptoms. Some of these side effects are severe, including suppression of white blood cells, pancreatitis and hepatitis, but other side effects could be mild, such as nausea, abdominal pain and fever. In the presence of a mild side effect during AZA use a trial of 6-MP is recommended because half of the intolerant patients tolerate a switch to 6-MP. A study was conducted to assess the long term outcomes of 6-MP treatment in patients with AZA intolerance. Fifty-two per cent of included patients tolerated 6-MP. In particular 6-MP was more tolerated in patients with hepatotoxicity and arthralgia/myalgia during AZA treatment. Less evident was the switching advantage in patients with hematologic and pancreatic toxicity^[8]. The role of thiopurines in CD is more important in the maintenance period, particularly in steroid-dependent CD, to induce early steroid sparing. A meta-analysis, including 7 placebo controlled trials, evaluated the efficacy of thiopurines in quiescent CD. AZA and 6-MP were effective in maintaining remission and inducing steroid sparing, but higher doses of AZA (2.5 mg/kg daily) were more effective than lower doses^[7,9]. According to these data the dosage of 2.5 mg/kg daily is suggested to optimize the treatment with thiopurines.

The efficacy of immunosuppressants was also evaluated in fistulizing CD. Eleven placebo controlled trials on the efficacy of immunosuppressants and antibiotics in fistulizing CD have been included in a meta-analysis. The analysis showed that both immunosuppressants and antibiotics were able to significantly reduce the number of open actively draining fistulas by at least 50% from the baseline. The incidence of severe adverse events was higher in patients treated with immunosuppressants than in patients treated with placebo ($P = 0.037$)^[10]. Regarding the maintenance treatment a questionable point is how long treatment with thiopurines should be continued. A meta-analysis showed that stopping thiopurine treatment increases the risk of relapse at 6, 12 and 18 mo. A clear benefit of continuing thiopurines for at least 18 mo was observed^[11]. In a trial of Lémann *et al.*^[12] patients who discontinued AZA after more than 3 years of efficacious treatment had a higher probability of relapse compared with those who continued it. According to the ECCO guidelines^[1] in patients treated with thiopurines as maintenance treatment, discontinuation may be considered after 4 years of remission. Benefit and risks of continuing them should be considered case by case.

A question related to the long term treatment with immunosuppressants is the reported high risk of lymphoma

and cutaneous malignancies. Patients with IBD treated with thiopurines alone or in combination with anti-TNF α had an increased risk of developing lymphoproliferative disorders^[13-15]. Many lymphomas associated to immunosuppressive therapy in IBD patients seem to be related to a loss of control of Epstein-Barr virus (EBV) infection. Young males seronegative for EBV are at risk for fatal forms of primary EBV infection, with lymphoproliferation. This incidence could be limited avoiding the treatment with thiopurines in this subgroup of patients^[16,17].

Hepatosplenic T cell lymphoma (HSTCL) is another rare, lethal form of lymphoma^[15]. Patients at risk are typically young men, treated for prolonged periods with thiopurines in combination with anti-TNF- α . The risk of HSTCL can be limited by avoiding prolonged combination therapy in young males. Concerning the risk of cutaneous malignancies, an increased risk of non-melanoma skin cancers (NMSC) has been reported in patients treated with thiopurines. This risk persists in patients previously exposed to thiopurines, suggesting a definite impact on carcinogenic events^[18-20]. In a retrospective study, 26403 patients with CD and 26974 patients with UC were matched with non-IBD controls. The incidence of NMSC was higher among patients with IBD compared with controls and thiopurine use was associated with NMSC, as was biologic use among patients with CD^[18]. Another prospective cohort study evaluated the incidence of NMSC among 19486 patients with IBD. The authors showed that ongoing thiopurine treatment and past thiopurine exposure were risk factors for NMSC. These patients should be protected against UV radiation and receive lifelong dermatologic screening^[19]. In a third study^[20], 9618 IBD patients were followed up and matched with 91378 controls. At the end of the study a diagnosis of basal cell skin cancer was made in 1696 individuals and a diagnosis of squamous cell skin cancer in 341 patients. IBD patients had an increased risk for basal cell skin cancer, compared with controls. Among patients with IBD, the use of thiopurines increased the risk of squamous cell skin cancer compared with controls. In order to explain the interindividual variability in efficacy and toxicity, the importance of measuring serum concentrations of thiopurine metabolites, 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine (6-MMP), has been recently proposed. Furthermore thiopurine methyltransferase (TPMT), that is one of the enzymes involved in the thiopurine metabolism, has been measured to predict the tolerability of thiopurines. A decreased TPMT activity has been related with myelotoxicity, whereas a high activity of TPMT has been related with thiopurine ineffectiveness. Low TPMT activity and high 6-TGN concentrations have been related to therapeutic success.

A prospective study was conducted to compare the 6-TGN levels in active IBD patients with those in patients in clinical remission. One hundred patients were included (41 with an exacerbation, 59 in remission). Twenty-six of the 41 patients (63%) with active disease and 24 of the 59 patients (41%) in clinical remission had

a 6-TGN level below the therapeutic cut-off level of 235 pmol/ 8×10^8 erythrocytes ($P = 0.04$)^[21]. Recent studies suggest that too low 6-TGN and too high 6-MMP nucleotide concentrations can be reversed by a combination therapy of allopurinol and thiopurines. In a study a dose-escalation of allopurinol was performed in 11 IBD patients with low 6-TGN and/or elevated 6-MMP concentrations, treated with AZA. Adequate 6-TGN concentrations were achieved with a combination of 25 mg allopurinol and 50 mg AZA in one patient and with 50 mg allopurinol and 50 mg AZA in nine patients. The 6-MMP concentrations were normalized immediately in all patients. The authors concluded that combination therapy with 50 mg allopurinol and 50 mg AZA daily is efficacious in IBD patients with inadequate thiopurine metabolite concentrations to optimize AZA therapy^[22].

The results of a recent prospective study do not support the capability of TPMT activity or 6-TGN to predict treatment outcome and no serum threshold value was identified to adjust the thiopurine dose. A total of 113 IBD patients treated with thiopurines were included. The TPMT activity was determined at inclusion (> 5 U/mL required) and thiopurine metabolites were periodically monitored. At the end of the study no cut-off point with worthwhile sensitivity/specificity was found. Eight patients showed thiopurine-related toxicity that could not be linked to TPMT activity or 6-TGN levels^[23].

Up to now the use of thiopurine metabolites has not been applied in clinical practice. A survey performed to evaluate the extent to which IBD gastroenterologists are utilizing thiopurine metabolism in practice showed that TPMT evaluation was performed by only 30% of gastroenterologists before AZA initiation. In patients on thiopurine therapy, 6-TGN and 6-MMP levels were determined by 54% and 44% of gastroenterologists respectively and 81% did not recheck metabolite levels after dose escalation or reduction^[24].

Methotrexate

Despite the wide use of thiopurines in the management of both CD and UC, approximately 20% of patients are intolerant and 30% are refractory to AZA/6-MP. An alternative immunosuppressant in these cases is MTX, an inhibitor of dihydrofolate reductase. Based on the results of 3 small studies^[25-27], where the efficacy of oral MTX was compared with placebo or 6-MP, MTX was considered ineffective in the treatment of active CD. A placebo-controlled trial^[28] compared the efficacy of intramuscular MTX (25 mg once weekly) with placebo. After 16 wk of therapy, 39% of patients were in clinical remission in the MTX group, compared with 19% of patients in the placebo group. The authors supported the efficacy of intramuscular MTX in active CD, although a higher rate of adverse events was reported with respect to placebo, including dyspepsia, alopecia, myelosuppression, increase in transaminase levels, abdominal pain, headache and arthralgia. To avoid some of these adverse events folic acid given routinely the day after MTX injection is recom-

mended. Furthermore, the teratogenic effect of this drug must be considered when it is started.

The efficacy of MTX in quiescent CD was observed in a trial^[29] in which MTX (15 mg weekly) intramuscularly was compared with placebo. After 40 wk of treatment, relapse occurred in 35% of patients treated with MTX and in 61% of patients treated with placebo. However in another two studies^[25,26] the superiority of MTX with respect to placebo, 5-ASA or 6-MP was not confirmed. Up to now the use of MTX in CD is confined to patients who are intolerant or non-responders to thiopurines.

Tacrolimus

Several studies, but not randomized controlled trials, evaluated the efficacy of oral or intravenous tacrolimus in the treatment of active luminal CD. Six of these studies, including a total of 70 patients, have been systematically reviewed^[30]. The duration of treatment varied from weeks to years. A total of 31 treated patients (44.3%) achieved a complete remission at the end of the studies. Eight studies evaluated the efficacy of tacrolimus in patients with perianal CD^[31-38]. Data were available for 49 patients with perianal disease who were treated with oral or intravenous tacrolimus and for 8 patients treated with topic tacrolimus. Among the patients treated with oral or intravenous tacrolimus 14 patients (28.6%) achieved remission and 19 patients (38.8%) achieved a partial response. Regarding the topic treatment, in a placebo controlled trial 8 patients with perianal CD were stratified according to ulcerating or fistulizing disease. A benefit in the ulcerating group was observed. No benefit was reported in the fistulizing group^[36].

Thalidomide

Thalidomide, originally used to treat morning sickness, had been withdrawn from the market because of its teratogenic effect. Subsequently immunomodulatory properties of thalidomide have been discovered as inhibitors of TNF- α synthesis. Thus in recent years its use was proposed for a few diseases including CD. No published randomized controlled trials on thalidomide efficacy in adult CD patients are available. In one trial^[39] the efficacy of its analogue lenalidomide was evaluated. In this study, 89 patients were included and randomized to receive 25 mg of lenalidomide daily, 5 mg of lenalidomide daily or placebo. At the end of the study the rate of clinical remission in both treatment groups was not significantly different from the placebo group. Other data come from uncontrolled studies. In a study^[40] 25 patients with luminal and fistulizing CD were treated with thalidomide. At the end of the study 6 out of 8 patients treated for luminal disease and 9 out of 11 patients treated for fistulizing disease achieved clinical response after a median follow-up of 12 mo. The 4 patients treated for both luminal and fistulizing disease had a fistula response and 3 of them had also a luminal response. The authors concluded that thalidomide was an effective treatment in patients with refractory luminal and fistulizing CD. Other encouraging

results come from the pediatric population. In a study^[41] 28 patients (young adults and children) with refractory moderate to severe IBD (19 CD, 9 UC) received thalidomide (1.5-2.5 mg/kg daily). Remission was achieved in 21 out of 28 (75%) patients (17 with CD, 4 with UC). In a recent small study^[42] 12 children, non-responders to previous drugs including AZA/6-MP, MTX and biologics, received thalidomide as rescue therapy. After 6 mo a significant improvement was observed in terms of symptoms, corticosteroid use, hospitalizations, laboratory values, fistula closure and surgery. Unfortunately a high rate of adverse reactions was reported during treatment with thalidomide, including peripheral neuropathy, dizziness and allergic reaction.

Based on these data the use of thalidomide can be proposed only in patients with active CD, refractory to conventional immunosuppressants and biologics, as rescue therapy. However given its teratogen nature and the high probability of side effects, its use for maintenance therapy is difficult to justify. However contraception should be recommended in woman with CD during thalidomide treatment. Up to now there are no recommendations for the use of thalidomide in CD. However it could have a role in the treatment of children with active CD despite immunosuppressive treatment.

Mycophenolate mofetil

MMF is an immunosuppressant largely used for the prevention of solid organ transplantation rejection. It is an antimetabolite similar to AZA and its use has been proposed in the management of autoimmune diseases, including psoriasis^[43], rheumatoid arthritis^[44] and IBD^[45,46].

A retrospective study^[47] assessed the efficacy and tolerance of MMF in 20 patients with CD who were intolerant or non-responders to AZA and/or MTX. The authors reported only 20% success with MMF and 25% intolerance. A study^[48] compared the efficacy of MMF with AZA in 45 patients with active CD, 15 treated with MMF and 30 with AZA over a period of 1 year. All patients who completed the 12 mo of treatment (77% AZA, 60% MMF) achieved remission but MMF patients had almost twice as many flare-ups (80% *vs* 47%) with respect to AZA patients. The authors concluded that both drugs were effective in inducing remission, AZA was more effective in maintaining remission but the onset of therapeutic effect was delayed less under MMF than under AZA. A randomized trial^[49] compared the efficacy of AZA plus corticosteroids with MMF plus corticosteroids in 70 patients with active CD. The authors observed a significant reduction in clinical activity with MMF plus corticosteroids compared to AZA plus corticosteroids. In patients with more severe CD, MMF plus corticosteroids caused a significant suppression of clinical activity earlier than AZA plus corticosteroids with few adverse effects.

Based on these data, although AZA is still the immunosuppressant of choice in the treatment of IBD, MMF may have a role for steroid-dependent patients refractory to other immunosuppressive therapies. Up to now there

are no recommendations for the use of MMF in CD.

biological therapies

In recent years the use of biologics has changed the management of IBD. The efficacy of anti-TNF α molecules (infliximab, adalimumab and certolizumab pegol) has been largely investigated in the treatment of active and quiescent CD, with good results^[50,51]. Up to now infliximab and adalimumab have been approved for the use in CD in many countries. These two anti-TNF α are both equal options in patients with moderate to severe steroid-refractory or steroid-dependent disease and in patients who are non responders or intolerant to immunosuppressants. Their use is also indicated in the case of complex perianal disease, in combination with surgical therapy. Furthermore, in presence of axial arthropathy (sacroileitis and ankylosing spondylitis) the efficacy of anti-TNF α treatment has been well established, as well as in the case of peripheral arthritis, non-responder to sulfasalazine^[1,2]. Certolizumab pegol is not approved in the European Union but in the United States and Switzerland it has the same indications as infliximab and adalimumab.

Two meta-analyses^[50,51] confirmed the efficacy of anti-TNF α antibodies in inducing and maintaining remission of luminal CD. Some studies evaluated the efficacy of these drugs in a subgroup of patients with steroid-dependent CD. Regarding infliximab, the study by Lémann *et al.*^[52] was planned to treat patients with steroid-dependent CD. These patients received infliximab 5 mg/kg or placebo at weeks 0, 2, and 6. All patients were treated also with AZA/6-MP for 52 wk. Among the 113 enrolled patients, the steroid-free remission rate was higher in the infliximab group than in the placebo group (75% *vs* 38%, $P < 0.001$ at week 12 and 40% *vs* 22%, $P = 0.04$ at week 52). Regarding adalimumab, data on steroid-dependent patients could be taken from a subgroup analysis of the CHARM study^[53]. At week 26, 19% of patients treated with adalimumab (40 mg every other week) achieved a sustained corticosteroid-free remission compared with 3% of patients treated with placebo ($P = 0.006$). At week 56, 29% of patients treated with adalimumab achieved a sustained corticosteroid-free remission compared with 5% of patients treated with placebo ($P = 0.001$). Recently the long term efficacy of adalimumab in steroid-dependent CD patients has been evaluated in a long-term open label extension of the CHARM study. After 4 years of follow up 16% of patients taking corticosteroids at baseline were in corticosteroid-free remission^[54]. A prospective observational study including 110 steroid-dependent CD patients reported clinical data on the efficacy and prognostic factors of response to adalimumab in corticosteroid-dependent CD patients. At week 6, 100 patients had a clinical benefit (91%), 50 of these (45.5%) were in steroid-free clinical remission, the other 50 (45.5%) achieved a clinical response. Nine patients (8.1%) did not achieve a clinical benefit and only one patient discontinued adalimumab due to intolerance. At the end of the follow-up (mean 14.6 \pm 10 mo, range

2-47 mo) 89 patients (80.9%) had a clinical benefit, 18 of these achieved a clinical response (16.4%), 71 were in complete remission (64.5%) and 11 discontinued adalimumab (10%) due to lack of efficacy or severe side effects. At multivariable analysis only a higher induction regimen was related to remission at week 6. At the end of the follow-up, none of the variables were associated with remission^[55]. All the 110 patients treated in this study have been followed up for a further 24 mo. At the end of the follow up (mean 38.6 \pm 8.6 mo) 54 patients (49%) were still on maintenance treatment with adalimumab with significant clinical benefit; 56 patients stopped treatment because of ineffectiveness (35), side effects (15) or mucosal healing (6). Mucosal healing was reported in 15 out of 60 patients who underwent colonoscopy (25%). At univariable analysis a lower induction regimen (80/40 mg) was associated with a best response to infliximab ($P < 0.001$, OR = 6, 95%CI: 1.01-35.91) while 160/80 mg induction regimen was associated with a lower risk of surgery with respect to 80/40 mg ($P = 0.04$, OR = 0.311, 95%CI: 0.969-0.998)^[56].

The advantage of biologics with respect to thiopurines is the more rapid effect. After 4 wk of treatment most of the treated patients show a clinical benefit^[57,58]. However from 17% to 21% of patients treated with infliximab develop antibodies directed against the murine sequences of infliximab molecule during the treatment, with immediate-type hypersensitivity reactions. Intravenous hydrocortisone premedication reduces antibodies to infliximab but some patients discontinue the treatment because of side effects, despite premedication and schedule treatment. Adalimumab, that is a recombinant human monoclonal antibody containing only human peptide sequences, has been proposed as an effective option in patients who had showed a previous allergic reaction to infliximab^[59]. Furthermore some patients treated with infliximab experience a loss of efficacy over time. In the Gain study^[60] 22% of CD patients with loss of response or previous intolerance to infliximab achieved remission with adalimumab. Based on these data, in the case of primary failures, loss of response or intolerance to infliximab, adalimumab can be used as a second line treatment. On the other hand the effectiveness of infliximab as a second line therapy after adalimumab failure has also been evaluated in CD patients. In a recent study 15 patients who discontinued adalimumab for loss of response (5), adverse events (3) or partial response (7), were treated with infliximab. After infliximab therapy all the patients who had discontinued adalimumab due to loss of efficacy or adverse events obtained a clinical response, but 2 of them developed adverse events. None of the patients who discontinued adalimumab due to partial response reached remission with infliximab^[61]. Up to now no trial has compared the efficacy and safety of infliximab and adalimumab in CD patients, thus the only factor that can guide the choice of one of the two biologics as first line therapy is the route of administration (subcutaneous for adalimumab and intravenous for infliximab). This should

be discussed with the patient and the choice of treatment should be made case by case. In the case of patients with primary failure to one anti-TNF α a switch to the other one should be considered. However, in the case of failure of one anti-TNF- α , before switching to the other one, an increase in dose or a decrease in the interval between infusions has been proposed. In a study, 54 out of 108 CD patients treated with infliximab received a dose intensification, defined as an increase in infliximab dose, a decrease in interval, or both, at 30 mo from initial infusion. At 30 mo 69.1% of patients were event-free from an interval decrease, 48.5% from a dose increase, and 45.7% from any dose intensification. Of the 54 patients who received a dose intensification, 75.9% were able to obtain and maintain a clinical response^[62]. In another prospective study 14 CD patients, initially responders to adalimumab, experienced a relapse. All patients were then treated with adalimumab 40 mg weekly for 12 wk before returning to adalimumab 40 mg every other week. Nine out of the 14 CD patients achieved a clinical response (1) or remission (8) 3 mo after reinstituting the standard dosage^[63]. Also, in the CHARM study^[53] the dosage of adalimumab could be escalated to open-label treatment with 40 mg weekly in patients with continued non response or recurrent flare. A total of 140 out of 854 enrolled patients completed the study on open-label adalimumab 40 mg weekly.

Two questionable points regarding biological therapy are: when to start and when to stop the treatment?

Some studies supported an early use of biologics in CD. In a randomized, controlled trial^[64] early combined infliximab and thiopurines treatment was more effective than a conventional step-up approach at 6 and 12 mo. Furthermore a subgroup analysis of the CHARM study^[65] showed a higher remission rate at 26 and 56 wk in patients with disease duration < 2 years with respect to patients with disease duration > 5 years. These data are not enough to confirm that an early treatment with biologics can improve patient outcomes. Therefore, a widespread early use of biologics in all CD patients cannot be recommended^[1,2] but in patient subgroups with a predicted disabling course^[66] (extensive disease, severe rectal disease, young age, severe perianal disease, steroid need at diagnosis) early introduction of biologics can be considered.

Regarding long term treatment, adalimumab was able to maintain remission for up to 4 years in patients who responded to induction therapy^[54] and long-term treatment with infliximab seems to have a safe maintenance efficacy^[67]. Despite these promising results the duration of biological therapy over 1 year is recommended only after a careful evaluation on a case-by-case basis and should be discussed with each patient^[1,2]. Some prospective studies^[68,69] showed that mucosal healing after biological therapy predicts a prolonged remission. Based on this observation a colonoscopy evaluation in patients receiving long-term anti-TNF- α therapy could guide the choice of treatment duration.

Recently pharmacokinetics and inter-individual variability of clearance and immunogenicity of biologics have

been evaluated to explain their efficacy and tolerability. Antidrug antibodies (ADA) impacted the clearance of infliximab and trough levels have been shown to correlate with clinical remission. In CD patients high trough levels of infliximab were associated positively with sustained clinical response, low C-reactive protein (CRP) levels and endoscopic improvement^[70]. In the SONIC trial, steroid-free remission at weeks 30 and 46 was more frequent in patients with high infliximab trough levels. Furthermore concomitant immunosuppressive therapy was shown to increase the trough levels of infliximab^[71]. In the AC-CENT trial a complete fistula healing was achieved in 64% of CD patients with high infliximab trough levels and in 25% of patients without detectable trough levels^[72]. In another study, in patients with infliximab levels below 12 mg/mL at mid infusion, a dose increase produced a response in 25 out of 29 patients and was more effective than changing to another anti-TNF- α agent^[73]. However the definition of therapeutic thresholds is still unclear. In a large series of 532 CD patients treated with infliximab from various trials a trough level of 3 mg/mL discriminated inflammatory activity^[74]. A cut-off of 3 mg/mL was associated with steroid-free remission in the SONIC trial^[71].

For adalimumab, pharmacokinetic-efficacy relations are less clear. In the CLASSIC trials correlations between adalimumab serum levels and remission rates were weak during induction and absent during maintenance therapy^[75]. In an observational cohort study^[76] a relationship between adalimumab serum trough levels and remission or response rates was not demonstrated.

Regarding the ADA evaluation, it seems to be the most important variable associated with low infliximab trough levels and low efficacy. The appearance of antibodies to infliximab (ATI) has been associated with loss of response and infusion reactions. In a study^[77], 73% of patients with loss of response to infliximab and all patients who developed infusion reactions showed ATI positive.

The occurrence of human antichimeric antibodies (HACA) was also inversely correlated with clinical response and positively correlated with infusion reactions^[78,79]. Premedication with glucocorticoids seems to reduce significantly the HACA levels^[77]. In a study it was observed that in 65% (11 out of 17) of patients initially positive for HACA, antibodies disappeared during maintenance treatment with infliximab but only in those with a clinical response^[80]. Furthermore the addition of immunosuppressants to infliximab therapy seems to restore the efficacy in patients positive for HACA^[81]. As for thiopurine metabolites up to now the utilization of biologic serum levels or ADA have not been routinely applied in clinical practice.

ULCERATIVE COLITIS

Immunosuppressant therapies

According to the ECCO guidelines^[3] patients with steroid-dependent UC should be treated with thiopurines.

The evidence to support the use of MTX in UC is insufficient. In patients with severely active UC, non-responder to intravenous steroids, the use of cyclosporine or infliximab is recommended.

Thiopurines

The efficacy of AZA in active UC was evaluated in two controlled trials, at the dosage of 2-2.5 mg/kg daily^[82,83]. A meta-analysis^[7] of these trials was conducted and a trend towards the benefit of AZA compared with placebo was observed but this did not achieve any statistical significance (RR = 0.85, 95%CI: 0.71-1.01, $P = 0.07$). In these two trials the efficacy of AZA was also investigated in quiescent UC. The meta-analysis of these trials showed a statistically significant benefit of AZA compared with placebo in preventing relapse (RR = 0.60, 95%CI: 0.37-0.95, $P = 0.03$)^[7]. In a study^[84] the efficacy of AZA was investigated in a subgroup of patients with steroid-dependent UC, comparing it to mesalamine. Seventy-two patients were randomised to receive AZA (2 mg/kg daily) or mesalamine (3.2 g daily), in addition to prednisolone (40 mg daily) for 6 mo. At the end of the follow up AZA was significantly more effective than mesalamine at achieving clinical and endoscopic remission with steroid sparing. More recently an observational cohort study was conducted in 42 patients with steroid-dependent UC treated with AZA (2-3 mg/kg daily) over a 3-year period. In this study AZA showed sustained efficacy for maintenance of clinical remission off steroids. Furthermore patients with earlier UC were those who most probably had sustained steroid-free remission at the end of 12 mo while on AZA^[85]. Considerations regarding the best dosage and the best duration of therapy with thiopurines are similar to the treatment for CD.

Methotrexate

Data on the efficacy of MTX in UC comes from small prospective studies where different doses and routes of administration have been used, with inconsistent results^[26,86,87]. A placebo-controlled trial showed no benefit of oral MTX at the dosage of 12.5 mg per week in UC patients^[86]. A study^[26] compared the efficacy of oral MTX at the dosage of 15 mg weekly with 6-MP (1.5 mg/kg daily) and mesalamine (3 g daily) in 72 steroid-dependent patients (34 UC and 38 CD). After 30 wk a significantly higher remission rate was observed in UC patients treated with 6-MP (78.6%) with respect to patients treated with mesalamine (25%) but no statistical differences were observed between patients treated with MTX (58.3%) with respect to patients treated with mesalamine. With regard to maintaining remission, after 76 wk UC patients treated with 6-MP presented significantly higher remission rates with respect to the other 2 groups. More recently a retrospective study^[88] evaluated the efficacy and safety of MTX in 131 patients with IBD (99 CD, 32 UC) intolerant or non-responsive to thiopurines. In UC patients clinical response occurred in 78% of patients refractory to AZA/MP and in 65% of pa-

tients intolerant to thiopurines. MTX was well tolerated in a majority of individuals. Based on these data there is insufficient evidence to support the use of MTX in UC as an alternative to thiopurines.

Tacrolimus

In patients with severely active UC not responding to intravenous corticosteroids a second line therapy with tacrolimus, as well as with ciclosporin or infliximab may be appropriate^[3]. A placebo controlled trial evaluated the efficacy of tacrolimus in 60 patients with moderate or severe UC. Patients were treated with a high concentration (10-15 ng/mL) of tacrolimus, a low concentration (5-10 ng/mL) of tacrolimus or placebo. At week 2, a clinical improvement was observed in 68.4% of patients treated with high concentration of tacrolimus and in 10% of patients treated with placebo ($P < 0.001$). Clinical remission was observed in 20% of patients treated with a high concentration. In the open label extension, 55.2% of all patients had a clinical improvement at week 10. The optimal target range appears to be 10-15 ng/mL. The incidence of side effects in the tacrolimus group was significantly higher than in the placebo group ($P = 0.043$). The most common event was finger tremor^[89]. A more recent placebo controlled trial was conducted on 62 patients with steroid-refractory UC. A response rate of 50% was reported in patients treated with oral tacrolimus compared to 13.3% in patients treated with placebo ($P = 0.003$) after 2 wk of treatment. Rates of mucosal healing were higher in the treatment group respect to the placebo group (43.8% *vs* 13.3%, $P = 0.012$), but the rates of clinical remission were not significantly different (3 out of 30 *vs* 0 out of 30, $P = 0.238$)^[90]. A retrospective review of 130 steroid refractory patients with moderate to severe UC showed that 94 patients (72%) had achieved remission after 3 mo of follow up and that the addition of thiopurines correlated significantly with remission rates^[91]. However another retrospective study on 32 refractory UC patients revealed that only 4 out of 30 steroid-dependent patients were able to discontinue steroids after a median follow-up of 29 wk and 12 out of 32 patients underwent colectomy^[92]. The efficacy of rectal tacrolimus ointment has been analysed in a small study on 8 patients with steroid dependent proctitis refractory to immunosuppressive and infliximab treatment. After 8 wk of therapy 6 out of 8 patients had achieved remission^[93].

A recent prospective study investigated the efficacy of topical tacrolimus in the treatment of refractor pouchitis. Ten patients with antibiotic-refractory pouchitis were treated for 8 wk with a tacrolimus enema. The mean pouchitis disease activity index score decreased from 15.9 ± 0.8 to 7.8 ± 0.8 during 8 wk of treatment ($P < 0.01$) but endoscopic healing was not achieved^[94].

Cyclosporine

A second line therapy with cyclosporine is indicated in the case of severe UC, which does not respond to 3-5 d of intravenous steroids. If no improvement is observed

within 4-7 d a colectomy is recommended. Two placebo controlled trials investigated the efficacy of cyclosporine in the treatment of severe UC^[95,96]. In the first study^[95] 30 patients with severe UC were randomized to intravenous cyclosporine at the dosage of 4 mg/kg daily or methylprednisolone at the dosage of 40 mg daily. After 8 d responders were treated with the same medication orally, in combination with AZA. After 8 d, 8 out of 15 patients (53%) who received methylprednisolone had a response compared with 9 out of 14 (64%) receiving cyclosporine. In non-responders, 3 out of 7 methylprednisolone patients and 1 out of 3 cyclosporine patients improved when both treatments were combined. At 12 mo, 7 out of 9 patients (78%) initially controlled with cyclosporine maintained their remission compared with 3 out of 8 (37%) initially treated with methylprednisolone.

In the second study^[96] 20 patients with severe UC, non-responders to 7 d of intravenous corticosteroids, were treated with intravenous cyclosporine (4 mg/kg daily) or placebo. Nine out of 11 patients (82%) treated with cyclosporine had a response within a mean of 7 d compared with 0 out of 9 patients who received placebo ($P < 0.001$).

Unfortunately the use of cyclosporine is associated with important adverse effects that are mainly dose dependent. A controlled trial investigated the additional clinical benefit of 4 mg/kg over 2 mg/kg intravenous cyclosporine in the treatment of severe UC. Seventy-three patients were included. After 8 d response rates were 84% in patients treated with 4 mg/kg and 86% in patients treated with 2 mg/kg. Furthermore short-term colectomy rates were 13% in patients treated with 4 mg/kg and 9% in patients treated with 2 mg/kg. The authors concluded that high-dose cyclosporine has no additional clinical benefit over low dose in the treatment of severe UC^[97]. Up to now 2 mg/kg daily has become the standard dose used in current clinical practice.

A study assessed the long-term colectomy-sparing effects and safety of cyclosporine in 71 patients with severe UC. Sixty out of 71 patients (85%) responded to intravenous cyclosporine and were discharged on oral cyclosporine. Of these 60 patients, 26 were transitioned from cyclosporine to 6-MP. Cumulative colectomy rates for the entire cohort were 39% at 1 year, 42% at 2 years, and 46% at 5 years. In the sub-group of patients maintained with 6-MP only one patient required a colectomy; whereas colectomy was carried out in 76% of patients who were not transitioned from cyclosporine to 6MP. In conclusion concomitant 6-MP therapy was associated with a reduced risk of colectomy (OR = 0.01, 95%CI: 0.001-0.09, $P < 0.0001$) on long-term follow-up. Side effects were noted in two-thirds of the patients, the majority of which were mild. The authors concluded that transition to oral thiopurines after intravenous cyclosporine was useful in preventing a future colectomy^[98].

Mycophenolate mofetil

As for CD, also for UC - data on the efficacy of MMF

come from small, uncontrolled, retrospective studies, in which patients with IBD and not only UC were included. A retrospective study^[99] evaluated the efficacy of MMF (1-2 g daily) in patients with IBD, only 7 of them with UC, treated over a 3-year period. Thirty-nine patients (32 CD and 7 UC), intolerant or non-responders to AZA, were identified. During the study period 40% of patients (a total of 16 patients, only 4 with UC) achieved remission and complete steroid withdrawal, 30% could not tolerate the drug, and 30% did not respond. An open-label prospective and uncontrolled 6 mo trial^[100] on the efficacy of MMF (2 g daily) in combination with steroids was conducted in 24 IBD patients (11 CD, 13 UC). Only 10 out of 24 patients achieved remission after 3 mo. All but one CD patient had relapsed by the end of the study. The authors concluded that MMF at the dosage of 2 g daily was unable to induce and maintain remission for a period of 6 mo in 23 out of 24 chronic active IBD patients. A retrospective study^[101] reported the results on the efficacy of MMF in 70 patients with IBD (51 CD, 19 UC) over a 5-year period. Seventeen of the 70 patients (24.3%) had a sustained steroid-free remission for 33 mo. Treatment with MMF was discontinued in 53 patients, 17 because of side effects and 36 because of non response to the treatment. A more recent study^[102] was conducted in 14 patients with IBD (9 CD, 5 UC) intolerant or refractory to conventional medical therapy who received MMF (500-2000 mg bid). Of the 11 patients who were not in remission at baseline, 7 out of 11 (63.6%) achieved remission after 8 wk. All 3 patients in remission at baseline maintained their remission. At 6 mo 64.3% patients were in remission. Of 12 patients followed for 12 mo, 8 were in remission (66.7%).

As for CD patients, MMF may be considered an alternative immunosuppressant therapy in steroid-dependent patients with UC, refractory to AZA/6-MP. Up to now there are no recommendations for the use of MMF in UC.

Biologic therapies

The use of anti-TNF α in UC patients is recommended in the case of severe UC that is refractory to steroids^[103,104] after exclusion of other causes of persistent symptoms such as coexistent cytomegalovirus or *Clostridium difficile* infection. Furthermore steroid-dependent UC patients, refractory or intolerant to thiopurines, can be treated with biologics. Also in patients with UC, as well as in CD patients, in the presence of axial arthropathy the efficacy of anti-TNF α treatment has been well established, as well as in the case of peripheral arthritis, not responding to sulfasalazine^[3].

A Cochrane meta-analysis on infliximab in moderate to severe UC, refractory to corticosteroids and/or immunomodulators, showed that infliximab was more effective than placebo in inducing clinical remission^[105]. Two placebo-controlled trials evaluated the efficacy of adalimumab in patients with moderately active UC despite conventional therapy^[104,106]. In one of these studies^[104]

adalimumab was compared with placebo in patients naïve to biologics. At week 8, clinical remission was observed in 18.5% of patients in the adalimumab group compared with 9.2% in the placebo group ($P = 0.031$). In the second study^[106] 40.3% of the included patients had been previously treated with infliximab. Clinical remission was achieved in significantly more patients receiving adalimumab than placebo at week 8 (16.5% and 9.3%, $P = 0.02$) and week 52 (17.3% and 8.5%, $P = 0.01$). The authors observed that the clinical remission rates in the adalimumab group were higher in patients who were naïve to anti-TNF α therapy at baseline. Other studies reported a benefit of adalimumab in patients with active UC previously exposed to infliximab with up to 27% of clinical remission in the short term^[107-109].

An Italian multicentre study reported data on the effectiveness of adalimumab in a cohort of 88 UC patients, retrospectively reviewed. Clinical remission rates were reported in 17%, 28.4%, 36.4% and 43.2% of the patients at 4, 12, 24 and 54 wk respectively. Twenty-two patients required colectomy. Clinical remission and low C-reactive protein at week 12 predicted clinical remission at week 54. Previous immunosuppressant use was associated with a lower probability of clinical remission at week 54 and with a higher rate of colectomy^[110].

Golimumab, a fully human monoclonal anti-TNF- α antibody, has been recently approved by the United States Food and Drug Administration for inducing and maintaining clinical remission in patients with moderate to severe UC. The efficacy of golimumab induction therapy has been evaluated in a trial on 1064 patients with UC. Patients were treated with golimumab at the dosage of 100 mg and then 50 mg, 200 mg and then 100 mg, 400 mg and then 200 mg, or placebo. The rates of clinical response at week 6 were 51% and 54.9% among patients treated with 200 mg/100 mg and 400 mg/200 mg golimumab, respectively, *vs* 30.3% among patients treated with placebo ($P \leq 0.0001$). The rates of clinical remission and mucosal healing were significantly greater in both golimumab groups than in the placebo group ($P \leq 0.0014$). The rates of serious adverse events were 6.1% and 3.0% in the placebo and golimumab groups, respectively^[111].

In a subsequent trial the efficacy of golimumab as maintenance therapy was evaluated. Patients who responded to induction therapy with golimumab ($n = 464$) were treated with placebo or golimumab (50 or 100 mg) every 4 wk through week 52. Patients who responded to placebo in the induction study continued to receive placebo. Non responders in the induction study received 100 mg of golimumab. At weeks 30 and 54, a higher percentage of patients who received 100 mg of golimumab were in clinical remission and had mucosal healing (27.8% and 42.4%) compared with patients who received placebo (15.6% and 26.6%; $P = 0.004$ and $P = 0.002$, respectively) or 50 mg of golimumab (23.2% and 41.7%, respectively)^[112].

Regarding the maintenance treatment, according to the ECCO guidelines^[3] in patients responding to anti-

TNF α both maintaining remission with thiopurines and anti-TNF α are considered appropriate. In patients with severe UC, responding to intravenous steroids, ciclosporin or infliximab, thiopurines should be considered to maintain remission. However, in patients responding to infliximab, continuing infliximab is also considered appropriate. The prior failure of thiopurines favours maintenance with anti-TNF- α therapy. According to the Italian guidelines^[2] 1 year scheduled treatment with infliximab is indicated in patients who have responded to infliximab induction. In patients who are thiopurine naïve, maintenance therapy with thiopurines may be a valuable option as maintenance treatment. The duration of therapy over 1 year should be evaluated case-by-case.

In severe UC infliximab is considered a second line salvage therapy before colectomy, such as ciclosporine^[2,3]. A small randomised controlled study showed that a single dose (5 mg/kg) of infliximab was an effective salvage therapy in patients with severe UC refractory to intravenous corticosteroids. Colectomy rates at 3 mo were significantly lower in patients receiving infliximab than placebo (7 out of 24 *vs* 14 out of 21, $P = 0.017$)^[113]. The authors observed that patients with a less active disease who were randomised after 5-7 d of intravenous corticosteroids seemed to benefit more than patients with more severe disease randomised at day 3. A retrospective Italian study^[114] then suggested that patients receiving a single infusion were more likely to require a colectomy at 2 mo than those who receive more infusions.

Other studies reported variable results^[115,116]. A placebo controlled trial showed a colectomy rate at 3 years of 50% in patients with severe UC treated with infliximab with respect to 76% in patients treated with placebo ($P = 0.012$)^[117].

The impact of preoperative infliximab use on the rate of surgical interventions and on post-surgical complications in patients with IBD was investigated in a meta-analysis. Twelve studies (8 on UC, 3 on CD and 1 on both UC and CD) were included in the analysis. In comparison with control groups, infliximab neither decreases the rate of colectomy nor increases the rate of infectious complications. However thrombotic events, ileal pouch complications, sepsis and anastomotic leaks were increased in patients treated with infliximab. On the other hand patients treated with biological therapies have a more severe disease and most of the time are refractory to other therapies. Thus it is possible that the postsurgical complications in these studies were associated with the severity of the disease and not with the treatment^[118].

A questionable point is what is the best second line rescue therapy in severe UC: infliximab or ciclosporine? Up to now no randomised trial showed clear advantages of one strategy over the other. A retrospective study compared 2 cohorts of patients receiving infliximab or ciclosporine as a rescue therapy. A lower immediate colectomy rate was reported in the ciclosporine group^[119]. At the end of the study the risk of colectomy was 11.2

(95%CI: 2.4-53.1, $P = 0.002$) at 3 mo and was 3.0 (95%CI: 1.1-8.2, $P = 0.030$) at 12 mo in infliximab treated patients, in comparison with cyclosporine treated patients.

Another retrospective study^[120] compared 2 historical cohorts of severe UC patients treated with cyclosporine (35 patients) or infliximab (30 patients). At 3 mo the colectomy rate was 28.5% in the cyclosporine group and 17% in the infliximab group ($P = 0.25$), at 12 mo the colectomy rate was 48% *vs* 17% ($P = 0.007$). At the end of the follow-up the colectomy rate was 60% *vs* 30% ($P = 0.04$). A high level of C reactive protein ($P = 0.04$), an extensive disease ($P = 0.01$) and no AZA treatment ($P = 0.001$) were related to the risk of colectomy.

In the CYSIF trial^[121] 111 patients with severe UC despite 5 d of intravenous corticosteroids were randomized to receive intravenous cyclosporine (2 mg/kg daily for 8 d) followed by oral cyclosporine (4 mg/kg daily) or infliximab (5 mg/kg at weeks 0, 2 and 6), followed by oral AZA. At day 7, 85% of patients responded to treatment in both groups. At day 98 treatment failure was reported in 60% of patients treated with cyclosporine compared with 54% of patients treated with infliximab ($P = 0.49$), with a colectomy rate of 18% in the cyclosporine group respect to 21% in the infliximab group ($P = 0.66$).

Regarding the evaluation of biologics serum levels or ADA in UC, in patients with UC infliximab serum levels were correlated with albumin serum levels^[122]. Patients with high serum albumin have a prolonged infliximab half-life and increased efficacy. Furthermore in UC patients the colectomy rate was significantly higher in the case of undetectable infliximab trough levels^[123]. In a post hoc analysis from the ACT I and ACT II studies higher infliximab concentrations were associated with an increased clinical remission and mucosal healing. The highest proportion of patients in clinical remission was observed in patients with serum levels of infliximab between 2.4 and 6.8 mg/mL at week 30^[124].

As for CD also in UC - biologics serum levels or ADA have not been routinely applied in clinical practice.

COMBINATION THERAPY IN IBD

Some studies supported the efficacy of combination therapy with biologics and thiopurines both in CD and UC. A trial compared the efficacy of induction treatment with infliximab plus AZA/6-MP to AZA/6-MP alone in steroid-dependent CD patients^[52]. At the end of the study, combination therapy was more effective than thiopurines alone. Furthermore a higher remission rate was observed in the subgroup of patients naive to AZA/6MP. In patients with previous thiopurines failure the difference between infliximab and placebo was not statistically significant. In another study^[70] combined immunosuppressive therapy with infliximab and AZA was able to obtain a higher steroid-free remission rate with respect to monotherapy with infliximab or AZA. Patients with moderate to severe CD were treated with infliximab, AZA or a combination therapy with the 2 drugs. After

26 wk 57% of patients assigned to combination therapy achieved a steroid-free remission, compared with 44% of patients assigned to infliximab alone and 30% of patients assigned to AZA alone. However the greater efficacy of combination therapy was observed mainly in patients with normal value of CRP and absence of endoscopic lesions at baseline.

Regarding UC, a recent trial^[125] suggested that in steroid-refractory patients with active UC a combination therapy with AZA and infliximab was more effective than either monotherapy. At week 16 clinical remission was achieved in 24% of patients receiving AZA, in 22% of patients received infliximab and in 40% of patients receiving combination therapy ($P = 0.032$ for combination therapy *vs* AZA monotherapy and $P = 0.017$ for combination therapy *vs* infliximab monotherapy).

These data are up to now insufficient to consider combination therapy efficient in all IBD patients. Furthermore, it is still being debated whether combined immunosuppressive therapy increases the long term toxicity. In clinical practice the use of combination therapy should be reserved for patients who do not respond to monotherapy.

The evidence of higher incidence of hepatosplenic T cell lymphoma in young men treated with combined immunosuppressive therapy^[126,127] should avoid prolonged combination therapy in young males. The recent observation that most of the lymphomas associated with immunosuppressive therapies in IBD patients were due to a loss of control of EBV infection leads to a recommendation of avoiding the use of thiopurines in patients seronegative for EBV^[128].

CONCLUSION

The use of immunosuppressive therapy, with conventional immunosuppressants or biologics, is indicated in the case of moderate to severe disease not responding to corticosteroids and in steroid-dependent IBD patients, as induction and maintenance treatment. Cyclosporine, infliximab and tacrolimus are considered a second line therapy in patients with severe UC not responding to 3-5 d of intravenous corticosteroids. The choice of biologics instead of immunosuppressants in the case of steroid-dependent IBD patients is guided by the severity of the disease, the presence of complex perianal disease in patients with CD or the concomitant extra-intestinal manifestations. Furthermore the recent observation that patients seronegative for EBV and treated with immunosuppressants have a higher risk of lymphomas, should lead to avoiding treatment with thiopurines in this subgroup of patients. The slow onset of action of thiopurines precludes their use as a single therapy in active IBD patients but they can be used in combination with corticosteroids. When treatment with thiopurines is chosen, adequate dosage and duration of therapy should be reached before evaluating their efficacy. The evaluation of serum concentration of thiopurine methyl transferase

activity and thiopurine metabolites could be useful for the management of the treatment but up to now it has not been routinely applied in clinical practice. The use of thalidomide and MMF is not recommended in IBD patients, the decision to use them might only be made on a case by case basis after failure of all other options including optimization of anti-TNF α therapies and surgical strategies.

Regarding biological treatment, dose intensification could be useful in the presence of incomplete response. In the case of primary failures to one anti-TNF α , a switch to the other one should be considered. In cases of intolerance to infliximab, the use of adalimumab as a second line treatment is recommended. Data on the efficacy of combination therapy are up to now insufficient to consider this strategy in all IBD patients. Furthermore the evidence of higher incidence of hepatosplenic T cell lymphoma in young men treated with combined immunosuppressive therapy means prolonged combination therapy should be avoided in these patients. As for immunosuppressants also for biologics - the evaluation of biologic serum levels and antibiologic antibodies could be useful for the management of the treatment but it is not routinely applied in clinical practice.

The final outcome of the treatment should be considered the clinical remission, with mucosa healing and not the clinical response.

Considering the evidence of high risk of cutaneous malignancies in patients treated with immunosuppressants and biologics for a long term period, lifelong sun protection and periodical dermatological screening should be recommended for these patients.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Preventing infective complications in inflammatory bowel disease

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Abstract

Over the past decade there has been a dramatic change in the treatment of patients with Crohn's disease and ulcerative colitis, which comprise the inflammatory bowel diseases (IBD). This is due to the increasing use of immunosuppressives and in particular the biological agents, which are being used earlier in the course of disease, and for longer durations, as these therapies result in better clinical outcomes for patients. This, however, has the potential to increase the risk of opportunistic and serious infections in these patients, most of which are preventable. Much like the risk for potential malignancy resulting from the use of these therapies long-term, a balance needs to be struck between medication use to control the disease with minimization of the risk of an opportunistic infection. This outcome is achieved by the physician's tailored use of justified therapies, and the patients' education and actions to minimize infection risk. The purpose of this review is to explore the evidence and guidelines available to all physicians managing patients with IBD using immunomodulating agents and to aid in the prevention

of opportunistic infections.

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Key words: Infection; Complications; Inflammatory bowel diseases; Immunosuppression; Anti-tumor necrosis factor agents

Core tip: In inflammatory bowel diseases (IBD) there is increasing use of immunosuppressives and the biological agents, which are being used earlier in the course of disease, and for longer durations. This, however, has the potential to increase the risk of opportunistic and serious infections in these patients, most of which are preventable. A balance thus needs to be struck between medication use to control the disease with minimization of the risk of an opportunistic infection. This outcome is achieved by the physician's tailored use of justified therapies, and the patients' education and actions to minimize infection risk. The purpose of this review is to explore the evidence and guidelines available to all physicians managing patients with IBD.

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INTRODUCTION

Patients with one of the inflammatory bowel diseases (IBDs), Crohn's disease (CD) or ulcerative colitis (UC), are at an increased risk of infection, which is partly inherent to the diseases themselves, but may also be due to the therapies used in their management. The pathogenesis of IBD is potentially secondary to an inappropriate in-

nate immune response to normal colonic flora and this may result in the lack of an appropriate immunological response to potential pathogens^[1]. In the more severe cases of IBD, patients may suffer from concurrent malnutrition and can need radical surgical procedures, which can further compromise the patients' immunological responses^[2]. The drugs required for disease control, such as the corticosteroids, immunological modulators like the thiopurines, methotrexate and cyclosporine, as well as the anti-tumor necrosis factor alpha (TNF α) medications, also have as their primary function the inhibition, and control, of immune system activity. Therefore, these can further reduce the immunological responses resulting in an increased risk of opportunistic infection.

The prevalence of opportunistic infections in IBD, however, is difficult to assess as this can vary markedly between countries but as they may result in mortalities within the IBD patient population their avoidance is of great importance^[3-6]. As an example, the background risk of tuberculosis (TB) in Spain is high at 21/100000, where it is considered endemic and the risk of infection can increase by up to 90-fold in IBD patients receiving an TNF α medication^[7]. By contrast there is a much lower background prevalence of TB in countries like the United States at 6.8/100000^[8] and 0.9/100000 in the non-indigenous Australian-born population^[9]. The risk to both the general population, and the immunosuppressed IBD patient, is thus vastly less in these countries and was demonstrated in an Australian and New Zealand study examining the prevalence of serious infections in IBD patients receiving a TNF α agent where not a single patient suffered from either primary, or reactivated TB, despite 3 patients receiving TB chemo prophylaxis due to a positive Quantiferon gold test prior to the initiation of TNF α therapy^[9].

While there is much concern regarding the TNF α drugs, as they may result in reactivation of granulomatous infections, particularly TB^[10], there is frequently less emphasis given to the other immunomodulating medications and whether they should also be regarded with caution, especially when used in combination with the TNF α therapies. The future of IBD medicine is, however, moving towards more biological medications (certolizumab, golimumab, natalizumab and vedolizumab) and the use of combination therapies. The risk to benefit ratio of these medications for the IBD patient thus needs to be continually assessed and monitored in order to give the best outcomes, much like the balancing act required to maintain IBD remission while minimizing the risk of cancers in these patients^[11].

Many infections have been associated with the use of the IBD medications, however, some may be specifically due to the mechanisms of action of individual medications^[12]. Patients on the thiopurine agents appear to be at greater risk of developing viral infections like cytomegalovirus (CMV), Epstein Barr virus (EBV) and varicella zoster virus (VZV), which is thought to be secondary to the effect of the thiopurine metabolites on T cells lead-

ing to the induction of apoptosis^[12]. By contrast, macrophage function is primarily affected in patients receiving a TNF α agent and it has clearly been documented that these medications reactivate TB and thus a meticulous screening program is required for these patients prior to undergoing these therapies^[10].

There is thus a definite risk of infections other than TB with the use of the IBD medications, but overall they appear to be uncommon. In the Australia and New Zealand study only 2.2% of the patient population receiving TNF α therapy suffered a serious opportunistic infection. Almost half of these cases, however, were on a combination of immunosuppressive therapies^[9]. This is similar to the findings of one of the first studies investigating opportunistic infection rates undertaken at the Mayo Clinic. This investigation demonstrated that the use of steroids, thiopurines and infliximab all impact on the rate of opportunistic infections in IBD. It noted that steroid use alone increased the risk by 2.6 fold (95%CI: 1.4-4.7) but this, however, increased further to 12.9 fold (95%CI: 4.5-37.0) when 2 or more of these drugs were used in combination^[13].

Despite how rare an opportunistic infection may be, however, the difficulty is in recognizing and treating them once they have occurred and the fact that they can result in significant morbidity and mortality. Prevention is thus certainly regarded as much better than cure in these situations. The prevention of opportunistic infections is, therefore, both the patient's and treating physician's primary goal and can be achieved through the use of multiple modalities that include vaccinations, chemoprophylaxis and education of the patients and clinicians. Each of these factors is vital for the successful implementation of appropriate guidelines for the best patient management^[14].

DEFINITION OF IMMUNOSUPPRESSION

An immunocompromised patient is someone in whom there is defective phagocytic, cellular, or humoral immunity, which leads to an increased risk of opportunistic infection and/or infective complications^[15,16]. While the presence of active IBD can itself lead to an increased risk of infections, independent of immunomodulating drugs, secondary to loss of the intestinal mucosal integrity, the IBD patient is not considered as immunocompromised *per se*. IBD patients are thus considered as being immunosuppressed primarily as a result of the therapy they receive and/or from the presence of malnutrition^[16,17]. The ECCO Consensus guidelines outline the various IBD therapies, which classify a patient as being immunocompromised and include the following: (1) treatment with steroids (prednisone or its equivalent of > 20 mg/d, or 2 mg/kg per day if < 10 kg, for 2 wk or more, and within 3 mo of stopping); (2) treatment with therapeutic doses of a thiopurine or discontinuation within the 3 mo preceding; (3) treatment with methotrexate or discontinuation within the preceding 3 mo; and (4) treatment with a TNF α agent or discontinuation within the preceding 3 mo^[16,17].

History	Examination	Investigations
Previous travel or living in tropical areas, future travel	Any active infections	Full blood count and differentials
Bacterial, viral or fungal infections	Dental examination	C-reactive protein
Risk of latent or active TB including country of origin, potential contacts, previous treatments	Pap smear	HCV, HBV and HIV serology (including HBV sAb if history of vaccination against HBV)
Immunization status		Stool cultures
		EBV, CMV and VZV serology
		Quantiferon gold assay, or tuberculin skin test (depending on local guidelines) for TB screening

Figure 1 The patient pre-inflammatory bowel disease therapy work up. HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; TB: Tuberculosis; CMV: Cytomegalovirus; EBV: Epstein Barr virus; VZV: Varicella zoster virus.

VACCINATIONS

Understanding the role of vaccination in the IBD population is crucial for the patient, specialist and primary care physicians involved in patient care. As advances in medical therapies lead to healthier patients with a better quality of life, focus must shift from treating infection to maintaining well-being in our patients by the prevention of disease. Vaccination is one of those vital, but frequently forgotten, areas in infection prevention. Patients with IBD are at risk of the same vaccine-preventable illnesses as the general population, and since most IBD patients will be diagnosed after they have completed their childhood immunization schedules, and most will require immunosuppression therapy at some stage in their lifetime, the opportunity should be taken to explore each patient's immunization status at the time of the diagnosis of their IBD^[15].

The institution of immunosuppressive and biological therapies also impact on which vaccinations a patient is allowed to receive and can also impact on the patient's response to vaccination with some studies demonstrating a lower response rate to vaccination once on these agents^[18,19]. There is usually only a small window of opportunity in which to vaccinate the patient prior to the institution of treatment with an immunomodulatory agent. This must be taken advantage of in order to achieve the best possible patient outcomes. To date it is clear that the vaccination rates in the IBD populations are suboptimal and these need to be improved^[20-23] and as there are now clear published international guidelines created to increase physician awareness of this issue and to improve vaccination rates and outcomes in the IBD population^[16].

What to do at diagnosis

At the initial diagnosis, or first presentation, of an IBD patient, a thorough history, clinical examination and panel of blood investigations should be performed prior to commencing any immunosuppressive, or biologic, therapy. This should include a history of previous, and current, infections including viral [VZV, herpes simplex virus (HSV), human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus, EBV and CMV], bacterial (TB, pneumococcal and urinary tract infections) and fungal infections. A detailed vaccination and travel history is also crucial in further determining what vaccinations need to be recommended, boosted or checked. Figure 1 summarizes the patient pre-IBD therapy work up and Table 1 summarizes the vaccination recommendations based on current guidelines and evidence.

Once a patient is on a immunocompromising medication, inactivated vaccinations only are recommended and these are suggested in guidelines for immunocompromised patients who do not have an increase risk of infectious complications^[24]. Live attenuated vaccinations need to be avoided in these patients (Table 1) as there is a risk that the administration of live vaccines to immunocompromised persons may result in adverse events, or vaccine-related disease, due to unchecked replication of the vaccine virus or bacteria. This is particularly noted for the measles-, mumps-, rubella^[25,26] and VZV-containing vaccines^[27] and for Bacille Calmette-Guérin (BCG) vaccine^[28,29]. The risk of disease, however, varies for different vaccines and for different individuals so caution is required for the use of vaccination in the setting of immunocompromise. In significantly immunocompromised persons the use of almost all the live vaccines are contraindicated.

The live attenuated vaccinations include yellow fever, oral polio, BCG, measles-mumps-rubella, typhoid Ty21a, VZV, live attenuated influenza virus and herpes zoster (Centre for Disease Control, 2009). Ideally a patient should not be receiving an immunomodulating medication for at least 3 mo prior to vaccination and in the case of steroids, the patient should avoid use for at least a month. If a live vaccine must be given to an IBD patient, the recommencement of an immunomodulatory medication should be withheld for at least 3 wk^[16].

RECOMMENDED VACCINATIONS - INACTIVE VACCINES

HBV vaccination

IBD patients who have less than 10 IU/L hepatitis B surface antibodies (anti-HBs) should be vaccinated against HBV according to the standard schedule (3 doses at 0, 1 and 6 mo) regardless of if they are immunosuppressed or not. When HBV vaccines are administered to a young healthy population, there is a > 95% protective seroconversion rate^[30-32]. Yet studies in the IBD populations have revealed much lower rates of detectable anti-HBs post vaccination (33%-36%)^[20,33], which could be attributable to

Table 1 Vaccines recommended in immunocompromised inflammatory bowel disease patients

Infectious disease	Vaccine type	Recommendation
Influenza	Inactivated trivalent virus	Recommended annually
Pneumococcal disease	23-valent purified capsular antigen	Recommended 5 yearly
Hepatitis B virus	Recombinant peptide	Recommended standard or double dose schedule
Human papilloma virus	Quadrivalent vaccine	In women according to local guidelines, standard schedule
Tetanus-Diphtheria	Toxoid	Recommended in vaccinated patients 10 yearly
Measles-Mumps-Rubella	Live attenuated	Contraindicated
Varicella zoster	Live attenuated	Contraindicated
Yellow fever	Live attenuated	Contraindicated
Cholera	Oral live	Contraindicated
	Oral killed	Use with caution
Poliomyelitis	Oral live attenuated	Contraindicated
	Injectable inactivated	Recommended
Meningococcal	Conjugated polysaccharide	Authorised yet not recommended
	C polysaccharide combined	Authorised yet not recommended
Tuberculosis	BCG live vaccine	Always contraindicated

BCG: Bacille Calmette-Guérin.

an older age group^[33] but also the use of biological therapy^[18,34]. The use of a thiopurine medication, however, appears to have no negative impact on the efficacy of HBV vaccination but this may need further investigation^[18].

Due to the significantly lower response rates to standard HBV vaccination in IBD patients, some studies have suggested a modified dosing regimen that doubles the standard antigen dose, given at 0, 1 and 2 mo. This was noted to result in 60% of the IBD patients having hepatitis B surface antigen levels > 10 IU/L^[18]. Comparison between this and the standard vaccination regime has been studied in IBD where 148 patients were vaccinated with either the standard, or double dose, protocol with an anti-HBs of > 10 IU/L considered a successful response^[35]. The seroconversion rate in the standard protocol group was 41% compared to a 75% seroconversion in the double dose protocol group. The advantage of the double dosing protocol was seen regardless of the use of immunosuppressive treatments and also was noted for achieving higher titres of anti-HBs with levels > 100 IU/L.

Considering the variability of HBV seroconversion, the best time to offer immunization is at the time of diagnosis prior to commencement of any immunomodulator therapy. Serological testing should then be undertaken after completion of the vaccination schedule (1-3 mo after the last dose) to determine if immunity was conferred^[16,31,32]. If the standard protocol fails to achieve seroconversion, an additional vaccine can achieve a successful antibody response in between 25%-50% of patients and a complete second three-dose course has been shown to be successful in between 40%-100% of patients in non-IBD population studies^[31,36].

Debate has also occurred around the ideal anti-HBs titre that should be reached post-vaccination in the IBD population. Post-vaccination anti-HBs titres of > 10 IU/L is considered to have conferred protection against infection in healthy subjects. This is long-term protection and relies on immune memory. In the immunocompromised patient, however, protection may be primarily reli-

ant on the amount of circulating antibody rather than the immune memory. Thus titres > 100 IU/L in the United Kingdom are now considered as the new cut-off point for the vaccination to be considered as successful in the immunocompromised patient^[32,37]. If these titres are not achieved after the 3-dose schedule, a 4th dose is then administered, or repetition of the full 3-dose series.

Influenza virus vaccination

Annual vaccination against influenza is recommended in IBD patients from the time of diagnosis^[16]. Immunosuppressed patients have a higher risk of complications secondary to the influenza virus and there is greater associated morbidity and mortality^[38]. The inactivated trivalent influenza vaccine comprises two type A subtypes (H1N1 and H3N2) as well as type B subtype. Studies have demonstrated mixed efficacy of the vaccine in the immunosuppressed population, mostly in paediatric IBD patients, which have demonstrated poor seroprotection results^[39-42]. However, more recent data in adult IBD patients have demonstrated adequate seroprotection rates without exacerbation of intestinal disease^[43]. Regardless of these findings, however, in most cases the immune response is adequate to warrant ongoing annual vaccination.

Pneumococcal vaccination

Streptococcus pneumoniae is the most common bacteria responsible for pneumonia and sepsis. IBD patients are also at increased risk of invasive pneumococcal sepsis^[17,20]. Vaccination with the 23-valent strain is thus recommended to be administered every 5 years in the IBD population^[16].

Again, as seen with other vaccines, effectiveness of this vaccine is diminished in patients on immunomodulating therapies, especially in combination, and therefore it should, ideally, be administered prior to commencement of such treatments^[44]. Considering that the vaccine comprises 23 antigens to mount an immune response too, some degree of protection can be achieved and, therefore, it is considered worthwhile.

Human papilloma virus vaccination

The human papilloma virus (HPV) is the most common sexually transmitted infection^[45]. This virus is oncogenic and can lead to cervical dysplasia with progression through to carcinoma^[46,47]. While data is lacking of a clear link with this risk being heightened and the use of immunosuppression, and biological therapies, there is a theoretical risk of HPV-associated tumours in prolonged and combination immunosuppressing IBD therapy. Vaccination against HPV in IBD is thus advisable in the appropriate populations (young women ageing from 12 years to 26 years old) according to the local guidelines^[16,48].

Tetanus and diphtheria

It is recommended that the general population should receive the tetanus, diphtheria and acellular pertussis vaccine every 10 years. This also holds true for patients with IBD. If the vaccination history is dubious then this should occur early within the IBD patient's course of treatment^[16,49].

LIVE ATTENUATED VACCINES

Measles-mumps-rubella vaccination

Childhood immunizations against measles, mumps and rubella should be included in the initial history taking of the new IBD patient. In most developed countries there is a low risk of acquiring these infections as an adult due to herd immunity^[50]. The evidence to support administration of the combined vaccine in patients prior to immunosuppressive therapy institution is also lacking and thus this is currently not a recommended vaccination by the ECCO guidelines.

Varicella vaccination

If patients with IBD have no history of having had varicella infection in childhood, serology should be checked and vaccination considered. Varicella infection in adults is more severe than in children, can be fatal and particularly severe if the patient is immunocompromised^[51]. Unfortunately, this vaccination is a live vaccine, and thus IBD patients who are varicella naïve and are already immunosuppressed should not receive it. If, however, the patient is known not to be immune to varicella prior to IBD therapy, a 2-dose schedule should be given at least 3 wk prior to commencing an immunosuppressive medication^[16]. Careful consideration of patients who might be at greater risk of varicella infection, such as children, teachers or health care workers, should guide the clinician in this decision.

CHEMOPROPHYLAXIS

Antibiotic prophylaxis has been a commonly used therapy in immunosuppressed patients to prevent opportunistic infections and the best example of this in IBD is for suspected latent, or active, TB. The TNF- α agents should be avoided in patients suspected of having latent, or ac-

tive, TB until treatment for TB has been commenced and has been in effect for at least 4 wk in order to avoid reactivation of TB, or according to local guidelines^[52-55].

Prophylaxis for *Pneumocystis jirovecii* with trimethoprim-sulpha-methoxazole^[12] should also be considered in patients on combination immunomodulatory regimes, usually when they are receiving the combination of 3 agents that includes steroids^[12,16,56], or in patients with low lymphocyte counts ($< 600/\mu\text{L}$)^[57]. Alternative agents are aerosolized pentamidine, dapsone and atovaquone^[58]. Data is lacking in this area and should be considered closely by the clinician on a case-by-case basis.

HSV

IBD patients with frequent and or severe recurrent HSV disease can be given oral anti-viral therapy to control these infections^[16]. Considering most infections with HSV are mild and self-limiting, chemoprophylaxis is not recommended in IBD patients commencing immunomodulators. If HSV infection, however, disseminates during immunosuppressing therapy, then treatment with high dose antivirals and cessation of immunosuppressors is recommended^[16].

HBV infection

HBV is a very common infection worldwide and is well known to reactivate in patients receiving immunosuppressive medications. This can result in significant morbidity and mortality, from liver function tests derangement through to fulminant hepatic failure and death unless anti-viral prophylaxis is given. This treatment strategy in preventing HBV flares is well established in patients with HBV-HIV co-infection and chronic HBV infected patients receiving systemic chemotherapy^[59] and is becoming increasingly important in IBD patients particularly on combination immunomodulatory therapy and on the biologics^[60]. There have been several case reports of fatal HBV flares in patients with IBD on immunosuppressant drugs^[61-64] drawing concerns that the TNF alpha drugs may be involved in regulating HBV replication^[65]. Patients who test positive for HbsAg should go onto anti-viral prophylaxis prior to commencing any immunosuppressive therapy. This is regardless of a detectable DNA viral load or not. Most recent guidelines suggest treatment with either entecavir or tenofovir over lamivudine due to fewer issues with developing viral resistance to these agents however most studies have been focused on lamivudine prophylaxis to date^[59].

HIV infection

Prior to highly active anti-retroviral therapy (HAART), immunosuppressive agents, especially the anti-TNF drugs were contraindicated in HIV-infected IBD patients. Now that viral replication can be controlled and immune reconstitution achieved with the use of HAART, both the immunosuppressive and biologic agents can be used to treat IBD in patients who have a CD4⁺ T lymphocyte count $> 500/\mu\text{L}$ ^[66,67]. In those patients who are not on

HAART, but require immunosuppressive or biologic agents, then initiation of HAART should take priority, especially if CD4⁺ T cell counts are < 500/ μ L.

EDUCATION

Patients need to be educated on how to recognize early symptoms of an opportunistic infection and to act quickly to get the required treatment if they are immunocompromised. Fever tends to be the most reliable, and sometimes the only, symptom for heralding the development of an opportunistic infection^[68] and IBD patients should always seek medical advice and/or review should they be experiencing this, especially in combination with other symptoms and the use of immunomodulator therapies. In these circumstances, a thorough history, examination, and septic work up should be performed by the clinician to help isolate the source of infection and guide therapy. Of course fever can also be a sign of a flare and thus this should also be considered.

If the suspicion for an infective cause of a fever in the context of digestive symptoms is high, then vigilance to exclude infection should be the priority, rather than the escalation of IBD therapy. Stool cultures that also examine for *Clostridium difficile* (*C. difficile*) toxin and ova, cysts and parasites should be performed. It must also be noted that a single stool culture may only exclude 66% of infections. Multiple stool cultures are thus recommended particularly for the excluding of *C. difficile* infection^[69]. *C. difficile* is an increasing problem in immunocompromised patients and current estimates suggest that approximately 10% of IBD patients will develop symptomatic *C. difficile* infection at some point during the course of their lifetime^[69]. This is important as it can lead to higher rates of colectomy and mortality.

If stool sample results are negative, an urgent colonoscopy or flexible sigmoidoscopy with colonic biopsies should be considered to assess for CMV colitis. This diagnosis is made on histological examination of biopsies taken at the interface of ulcers. Serum CMV PCR can also be performed but is not specific for active CMV disease^[70].

CONCLUSION

In an era of increasing use of immunosuppressing medications in IBD and for longer durations, together with advocacy of the use of combination therapy, patients and their doctors need to be more vigilant about prevention and detection of opportunistic infections. IBD patients are at the same risk for vaccine-preventable illness as the general population. As IBD therapy can affect vaccine efficacy, then vaccination should be considered early in the course of disease and ideally prior to the commencement of any immunocompromising medication. The challenge for doctors is to balance the medical management of IBD, knowing the risks of individual therapies, and recognizing that prevention of opportunistic infections is as

of equal importance. This usually requires the best use of one of the most precious commodities a doctor has with their patient, time.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Treating inflammatory bowel disease by adsorptive leucocytophoresis: A desire to treat without drugs

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Abstract

Ulcerative colitis and Crohn's disease are the major phenotypes of the idiopathic inflammatory bowel disease (IBD), which afflicts millions of individuals throughout the world with debilitating symptoms, impairing function and quality of life. Current medications are aimed at reducing the symptoms or suppressing exacerbations. However, patients require life-long medications, and this can lead to drug dependency, loss of response together with adverse side effects. Indeed, drug side effects become additional morbidity factor in many patients on long-term medications. Nonetheless, the efficacy of anti-tumour necrosis factors (TNF)- α biologics has validated the role of inflammatory cytokines notably TNF- α in the exacerbation of IBD. However, inflammatory cytokines are released by patients' own cellular elements

including myeloid lineage leucocytes, which in patients with IBD are elevated with activation behaviour and prolonged survival. Accordingly, these leucocytes appear logical targets of therapy and can be depleted by adsorptive granulocyte/monocyte apheresis (GMA) with an Adacolumn. Based on this background, recently GMA has been applied to treat patients with IBD in Japan and in the European Union countries. Efficacy rates have been impressive as well as disappointing. In fact the clinical response to GMA seems to define the patients' disease course, response to medications, duration of active disease, and severity at entry. The best responders have been first episode cases (up to 100%) followed by steroid naïve and patients with a short duration of active disease prior to GMA. Patients with deep ulcers together with extensive loss of the mucosal tissue and cases with a long duration of IBD refractory to existing medications are not likely to benefit from GMA. It is clinically interesting that patients who respond to GMA have a good long-term disease course by avoiding drugs including corticosteroids in the early stage of their IBD. Additionally, GMA is very much favoured by patients for its good safety profile. GMA in 21st century reminds us of phlebotomy as a major medical practice at the time of Hippocrates. However, in patients with IBD, there is a scope for removing from the body the sources of pro-inflammatory cytokines and achieve disease remission. The bottom line is that by introducing GMA at an early stage following the onset of IBD or before patients develop extensive mucosal damage and become refractory to medications, many patients should respond to GMA and avoid pharmacologics. This should fulfill the desire to treat without drugs.

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Key words: Inflammatory bowel disease; Myeloid lineage leucocytes; Adsorptive granulocytes/monocytes apheresis; Corticosteroid sparing effect; Complement activation fragments; Treating inflammatory bowel disease without drugs

Core tip: The efficacy of anti-tumour necrosis factor- α biologics has validated the role of inflammatory cytokines in the exacerbation of inflammatory bowel disease (IBD). However, inflammatory cytokines are released by patients' own cellular elements including myeloid lineage leucocytes, which in patients with IBD are elevated with activation behaviour. Accordingly, these leucocytes appear logical targets of therapy and can be depleted by adsorptive granulocyte/monocyte apheresis (GMA). Therefore, in patients with IBD, there is a scope for removing from the body the sources of pro-inflammatory cytokines, and this should fulfill the desire to treat without drugs. Therefore, by introducing GMA at an early stage following the onset of IBD or before patients develop extensive mucosal damage, many patients should respond to GMA and avoid pharmacologics.

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INFLAMMATORY BOWEL DISEASE

The idiopathic inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's diseases (CD) is an immune disorder characterized by inflammation of the gastrointestinal tract in genetically susceptible individuals when exposed to environmental risk factors^[1]. Therefore, IBD has long been thought to have a genetic basis and involves response of the immune mechanisms to certain environmental agent(s). The development of IBD among monozygotic twins and an increased incidence in countries undergoing rapid Westernization highlight the relevance of genetic predisposition together with environmental factors in disease pathogenesis and incidence^[2]. What all these mean is that in patients with IBD, the intestinal immune profile becomes dysregulated showing exaggerated response to triggers that would have been insignificant in an individual without genetic susceptibility factor.

However, it is clinically relevant to bear in mind that whereas UC is confined to the colon and the rectum (large intestine), CD may affect any part of the gut from the mouth to the perianal region. Most commonly CD affects the distal ileum and the colon; up to 65% of CD patients present with small intestinal involvement. Both UC and CD are debilitating chronic health disorders that afflict millions of individuals throughout the world with symptoms, which impair function and quality of life.

Further, the human intestine is host to thousands of bacterial species, collectively referred to as the intestinal microbiota or microflora^[3]. While it is understood that the presence of this microbiota is essential for human

health, this relationship may become unbalanced, and translate into development of UC or CD^[3,4]. Accordingly, both clinical and experimental observations indicate that IBD flare-ups are triggered by a combined loss of the so-called intestinal barrier function and a dysregulated immune response to the intestinal microbiota^[3,5].

A multitude of clinical manifestations represent the expression of IBD. These include diarrhoea, rectal bleeding, abdominal discomfort, fever, anaemia, and weight loss. Both UC and CD tend to run a remitting-relapsing course affected by diverse factors mentioned above. The severity of UC is often presented by a clinical activity index (CAI), which is described by several authors^[6-9]. Another, but complementary parameter is endoscopic activity index (EI), which is a true reflection of mucosal damage or otherwise mucosal remission^[10] calculated according to Rachmilewitz^[9] or the Mayo scoring method^[7]. Further, disease activity in CD, which is the other phenotype of IBD is assessed by the well known CD activity index (CDAI)^[11]. A CDAI of < 150 reflects remission. The authors of this review (Tanaka T, Ohmori T, Sawada K, Yamamoto T, Hanai H) are gastroenterologists and routinely evaluate patients by both CAI and endoscopy/colonoscopy with greater reliance on endoscopic evaluation^[10]. Therefore, in this article, our endeavours were supported by the diagnostic power of colonoscopy to identify patients with an active flare of IBD who were identified as the most likely responders to selective, but therapeutic removal of circulating myeloid lineage leucocytes (granulocytes and monocytes/macrophages) by extracorporeal adsorption as a non-pharmacologic treatment intervention. This strategy is known as GMA, which stands for adsorptive granulocyte and monocyte apheresis, described later. In Figure 1 colonoscopic photographs from the colonic mucosa of a healthy human subject and from patients with UC are presented. The mucosa is the surface through, which nutrients and water from the food in the intestine are absorbed into the blood stream. Accordingly, healthy mucosa has a well developed vascular network for adequate absorption of water and nutrients. However, in patients with IBD, the vascular patterns may become invisible or lost due to inflammation or ulcers, seen in Figure 1. As the mucosal layer is involved in the absorption of nutrients and water from the gut, during severely active IBD, absorption of nutrients and water is seriously impaired. Extensive and deep ulcers together with near total loss of the mucosal tissue are not uncommon in patients with severe IBD even in the presence of conventional medications. This condition is debilitating, the patients may suffer from weight loss. For example unabsorbed food and water will pass as watery diarrhoea, or bloody diarrhoea (due to bleeding ulcers). Patients with deep ulcers and extensive loss of the mucosal tissue are not likely to respond to any drug based medication or even to therapeutic depletion of myeloid leucocytes by GMA, they have fulminant UC (disease persists in the presence of optimal medication) and often must opt for resection of the affected gut segment. Needless to say that only an

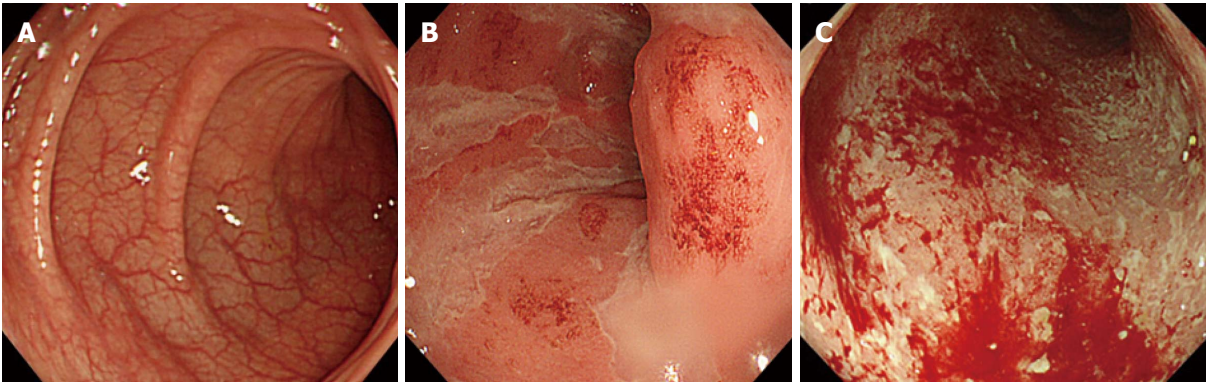


Figure 1 Colonoscopy images. A: Colonoscopy showing normal mucosa with visible vascular patterns in a healthy, non-inflammatory bowel disease (IBD) individual; B: During strong inflammation in a patient with IBD, visible vascular patterns are lost; C: Inflammation can lead to ulcerated mucosa and contact bleeding. Both mucosal inflammation and ulcers lead to inadequate absorption of water and nutrients from the gut. The affected patients may lose weight and become anaemic due to bloody diarrhoea, which if untreated can be very debilitating.

initial diagnostic colonoscopy can identify such patients as non-responders to drug based interventions so that the patient can opt for surgical intervention at an early stage. This should significantly shorten morbidity time and help to avoid futile use of medical resources.

THERAPEUTIC OPTIONS FOR PATIENTS WITH IBD

However, currently available medications aid in the induction and maintenance of remission by targeting various points along the disordered immune pathway implicated in IBD flare ups^[12-17]. Despite these seemingly advances, knowledge on the precise cause of IBD is inadequate at present. Accordingly, up to now drug therapy has been empirical rather than based on a sound understanding of disease aetiology. While drug therapy initially appears effective in the majority of patients, it comes at the cost of serious side effects^[18-20], which add to the disease complexity. First-line medications for exacerbation of IBD include 5-aminosalicylic acid or sulphasalazine in combination with a corticosteroid together with azathioprine (or 6-mercaptopurine) and nutritional support for some patients^[6,16,17]. Treatment failure in patients with severe disease has often been an indication for surgical intervention in many steroid refractory patients^[14,21] although in recent years, cyclosporin A (CsA) has been introduced for corticosteroid refractory UC^[8,9,15]. Despite being moderately effective in this clinical setting in reducing surgery rate, there remain serious concerns over long-term efficacy and toxicity of CsA^[20]. The development of new anti-tumour necrosis factor (TNF)- α antibodies like infliximab and adalimumab represent progress, but there is concern about safety and long term efficacy of biologics. However, this is not to say that drugs have no place in the treatment of IBD. In fact, no one can deny the role of medicines in the elimination of most diseases that our ancestors were left defenseless against. Instead, in this review, our endeavour has been to present clinical evidence and thera-

peutic outcomes supporting the idea that many patients with IBD can be treated without drugs, but by targeted apheresis.

CYTOKINES AND INFLAMMATORY BOWEL DISEASE

As stated above, the precise cause of IBD is not fully understood at present, and currently available medications do not eradicate the fundamental cause. Therefore, it should be logical to identify known exacerbating factors as therapeutic targets. In line with this thinking, patients with active IBD show elevated cytokine expression, aberrant antigen-antibody complexes, T-cell anomalies, and an increased numbers of granulocytes and monocytes/macrophages (myeloid lineage leucocytes)^[22,23], which show activation behaviour^[24,25], and prolonged survival^[26]. The interaction between neutrophils and macrophages is thought to induce a state of chronic inflammation, which contributes to the perpetuation of IBD. Additionally, the chronic nature of IBD means that afflicted individuals need life-long drug therapy and this can lead to refractoriness^[18] and drug-related adverse side effects^[19]. However, it is now widely known that IBD is exacerbated and perpetuated by the so-called inflammatory cytokines including TNF- α , interleukin (IL)-1 β , IL-23 and others^[27-29]. Accordingly, patients with active IBD respond to anti-cytokine antibodies, notably, to anti-TNF- α biologics^[30,31] albeit having known serious side effects^[32-34]. Nonetheless, the clinical response to anti-TNF biologics has validated the role of this cytokine as an exacerbating factor in dysregulated immune profile. Further cytokines like TNF- α , IL-1 β and others are produced by patients' own cellular elements, notably by the myeloid lineage leucocytes^[24,25,35,36]. Hence, elevated circulating myeloid lineage leucocytes appear logical targets of therapy by selective leucocytapheresis we introduced as GMA above. In this article, we have reviewed the latest developments in therapeutic leucocytapheresis to treat IBD with the Adacolumn GMA.

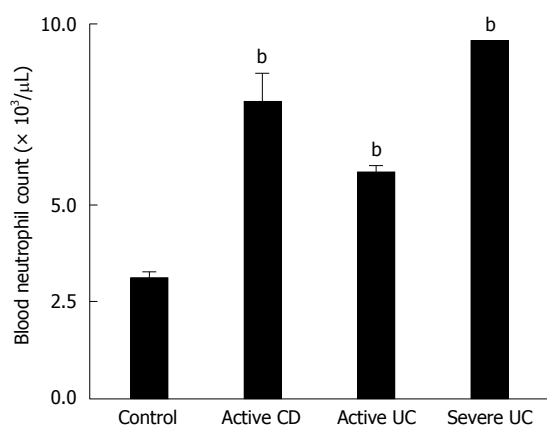


Figure 2 Blood neutrophil (granulocyte) counts in healthy controls and patients with inflammatory bowel disease. This figure shows a very marked elevation of neutrophils during active inflammatory bowel disease (IBD). Additionally, in patients with IBD, neutrophils show activation behaviour and increased survival time in the circulation^[26,36] as well as within the mucosal tissue^[26]. ^b $P < 0.01$ vs control group. UC: Ulcerative colitis; CD: Crohn's disease.

EVOLUTION OF APHERESIS AS A THERAPEUTIC PRACTICE

The word “apheresis” means to take away or to remove, clean from disease causing elements. In the modern time, apheresis means an extracorporeal medical technique for selective removal of components of the blood to achieve a therapeutic effect. Thus apheresis is an extracorporeal therapy. In classical sense, the most basic practice in apheresis involves passing of a patient's blood through a medical device where removal of components takes place; it is a kind of bloodletting. In the early 20th century, dialysis^[37], and centrifugation^[38] were introduced for selective depletion of soluble elements^[37] or blood cells^[38] to achieve therapeutic effects. Even in today's biologic based therapy targeting a pathologic cytokine, chemokine or an adhesion molecule, apheresis is considered as a non-drug strategy to manage diseases for which there is no effective drug based medication like removing toxic substances from the plasma, which threaten well-being^[39,40]. Strategies that selectively remove plasma components or blood cells as therapeutic interventions have generally been safe, and not associated with any major side effect in a significant number of patients^[39-41]. Recently, apheresis has been considered as an effective alternative to drug therapy or as an adjunct to conventional medications to enhance drug efficacy with potential to reduce standard medication dosage like the GMA trials in patients with IBD where corticosteroids were avoided or were tapered to a minimum^[41-44].

However, today's selective removal of constituents of circulating blood to achieve a therapeutic effect by apheresis is reminiscent of the rather crude technique of bloodletting (phlebotomy) and its therapeutic application at the time of Hippocrates (460-377 BC) in Ancient Greece. Bloodletting was widely practiced as a major therapeutic intervention in the Ancient civilization of Egypt from where it expanded to Ancient Greece and

other parts of the world. The perception then was that disease reflected presence of disease-causing substances in the blood and bloodletting was to expel the pathologic agents. Bloodletting was routinely and extensively practiced for diseases like inflammation, fever, hypertension, and other undiagnosed diseases, its most high profile patient was the first US president, George Washington (reviewed in ref^[45]). Bloodletting as a major medical practice was subsequently popularized by Claudius Galen (129-203 AC), a Greek physician who practiced in Rome and became a well respected authority in medicine for over 1500 years. In the late 19th century, bloodletting under a more modern name, phlebotomy was popularly used to treat conditions like haemochromatosis, polycythaemia vera and others. It may just be the folly of the past, but it is difficult to imagine that this procedure would have been so widely practiced for such a long time if it had not been associated with efficacy.

Even in this era of modern medicine and if we consider IBD, and innovations in biologic therapy, it is clinically relevant to bear in mind that drug therapy by its very nature, involves adding a foreign substance or substances to the body system and although initially may be effective, but potentially can lead to the disease becoming drug dependent or drug refractory (body's reaction to a foreign substance). Hence, a therapeutic strategy based on a non-drug intervention, a correction or support of body's natural processes like GMA^[41-53], if effective has an unrivalled advantage over drug based medication.

TARGET LEUCOCYTES FOR THERAPEUTIC GMA

For an extracorporeal intervention like GMA to be a novel non-drug therapeutic option, it should selectively deplete leucocytes, which in patients with IBD are thought to contribute to the disease pathogenesis. For example, patients with active IBD are found to have compromised lymphocytes^[42,47,54] in the presence of elevated and activated granulocytes and monocytes/macrophages^[46,48] seen in Figure 2. Additionally, certain sub-populations of lymphocytes like the CD4(+)CD25(+) phenotype, known as the regulatory T-cells (Treg) have essential immunoregulatory function and therefore, are indispensable to the host^[55-61]. Based on these understandings, the Adacolumn for GMA is designed to spare lymphocytes, seen in Figure 3, and boost Treg phenotype^[48,61]. The column is filled with specially designed cellulose acetate beads of 2mm in diameter as leucocytapheresis carriers^[22]. As shown in Figure 3, the carriers remove from blood in the Adacolumn most of the granulocytes, monocytes/macrophages together with a significant fraction of platelets^[23,48]. Surprisingly, the procedure has been associated with a sustained increase in absolute lymphocyte counts following a course of GMA^[22]. The increase in lymphocyte counts includes the Treg phenotype, CD4(+)CD25(+)^[61]. The mechanisms for sparing lymphocytes are briefly described here and illustrated in Figure 4. Patients with IBD may

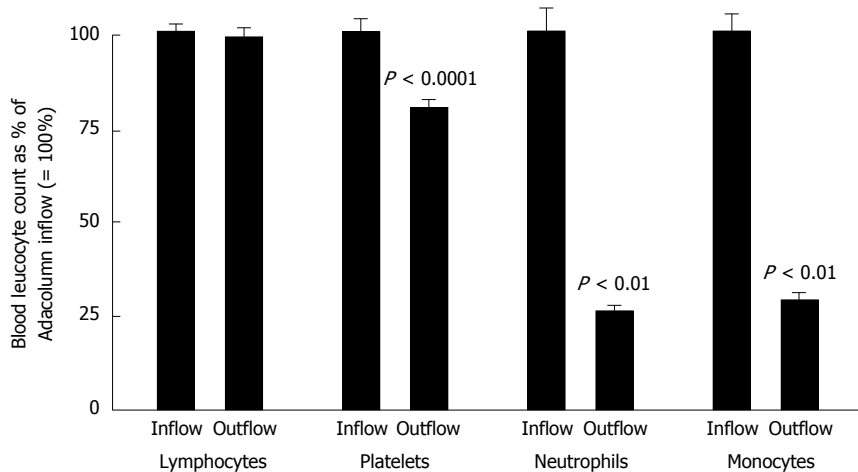


Figure 3 Selective depletion of myeloid lineage cells (neutrophils, monocytes and platelets) by adsorptive granulocyte and monocyte apheresis with the Adacolumn in patients with inflammatory bowel disease. In each case, the column inflow count was expressed as 100% and the column outflow count (return to patient) was expressed as a percentage of the inflow count taken 30 min (peak fall) during a 60-min granulocyte and monocyte apheresis (GMA) session. The data serve as a solid basis for the argument that GMA selectively depletes granulocytes, monocytes/macrophages together with a significant fraction of platelets, but spares lymphocytes^[22,23,48,117].

have immune complexes (IC) in their plasma^[23,62,63]. Cellulose acetate adsorbs immunoglobulin G (IgG) and IC from the plasma^[23,62,63]. Upon adsorption, the binding sites on IgG and IC become available for the fragment crystallizable gamma (Fcγ) receptors (FcγRs) on myeloid lineage leucocytes^[23,62,63]. Further, cellulose acetate with adsorbed IgG and IC generates complement activation fragments including C3a and C5a^[23,63]. The opsonins C3b/C3bi and others derived from the complement activation fragments also adsorb onto the carriers and serve as the binding sites for the leucocyte complement receptors, CR1, CR2, CR3 (Mac-1, CD11b/CD18). Hence, leucocyte adsorption to the GMA carriers in the Adacolumn is governed by the opsonins, FcγRs and the leucocytes complement receptors^[23]. The expressions of these sets of receptors are common features of myeloid lineage leucocytes. Lymphocytes are not known to express complement receptors except on small subsets of B, T and natural killer (NK) cells. Similarly, FcγRs are not widely expressed on lymphocytes except on small populations of CD19⁺B cells and CD56⁺NK cells^[62]. These basic phenomena proceed well on the carriers and lend GMA selectivity (Figure 4). Further, to our knowledge, there is no published data showing elevated peripheral lymphocytes in patients with active IBD and in one of the best controlled studies on lymphocytapheresis in IBD, Lerebours *et al.*^[64] selectively depleted circulating lymphocytes in patients with CD. At the end of an 18-mo follow-up, the clinical outcome in the lymphocytapheresis group was 21% inferior to that of the control group. This well planned controlled clinical trial^[64] rules out therapeutic benefit from removing lymphocytes in patients with IBD.

CLINICAL EFFICACY RATES FOR GMA IN UC PATIENTS

Hitherto, a large number of articles mostly from Ja-

pan^[65-77], and Europe^[78-91], but also from the United States^[92-96] have described the efficacy of GMA in patients with IBD. The clinical application of GMA with the Adacolumn began following a pioneering multicentre clinical trial by Shimoyama *et al.*^[41] described below under steroid sparing effects of GMA. Since then, GMA has been widely applied in Japan and in the European Union (EU) countries to treat patients with IBD. Efficacy rates have been both striking^[42-44] as well as disappointing^[92,93], reflecting patients' diverse demographic features prior to GMA. Further, the authors of this review regularly apply GMA to treat patients with IBD for longer than a decade. Based on our experience, the best responders are first episode cases who are often drug naïve and have a short duration of IBD^[52], followed by steroid naïve patients^[42,50,53,75]. Efficacy rate in first episode cases has been as high as 100%^[52] and in steroid naïve cases over an 85%^[42,50]. Likewise, recently, Yokoyama *et al.*^[74] reported that the most appropriate time to apply GMA therapy in patients with UC was immediately after a clinical relapse. The authors found that the best responders were those with a short duration of active disease^[74]. However, patients with deep mucosal lesions and extensive loss of the mucosal tissue at the lesion sites^[52,53,82,93] together with those who have a long duration of active UC and exposure to multiple conventional drugs including corticosteroids when the UC has become refractory to medications are not likely to benefit from GMA^[49,52,92,93]. These are clinically relevant findings because patients who responded to GMA have a better long-term clinical outcome by avoiding corticosteroids during their first active UC phase^[70], while knowing GMA non-responders should help to save medical cost by avoiding futile application of GMA. Figure 5 shows clinical remission rates in typical cohorts of patients with active UC. As shown, the remission rate for patients with the first UC episode has been 100%^[52]. All these first episode cases were steroid naïve with a short (< 4 mo) duration of UC.

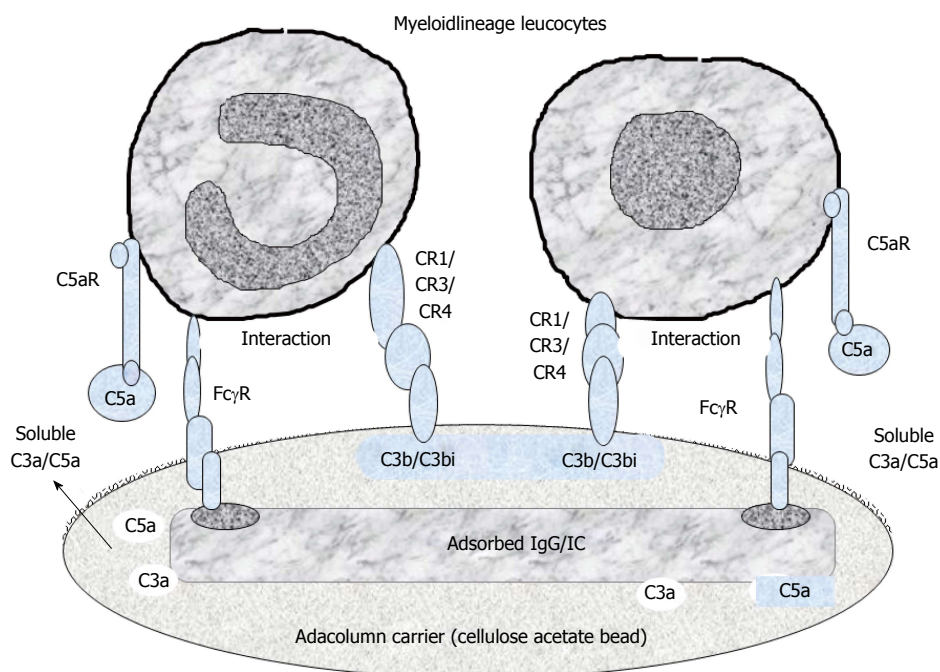


Figure 4 A tentative illustration of the mechanisms, which mediate the selective adhesion of myeloid lineage leucocytes to the granulocyte and monocyte apheresis cellulose acetate carriers. The first event is adhesion of plasma immunoglobulin (IgG) and immune complexes (IC) to the carriers, which serve as the binding sites for the fragment crystallizable gamma receptor (Fc γ R) on myeloid leucocytes. Then complement activation (helped by IgG and IC) generates C3a, C5a, C3b/C3bi fragments. Of these C3b/C3bi (opsonins) adsorb onto the carriers and serve as the binding sites for the complement receptors (CR) on myeloid leucocytes. Adhesion of leucocytes results in the release of interleukin-1 receptor antagonists, hepatocyte growth factor, soluble tumour necrosis factor receptors, all with therapeutic effects (Figure 13). Modified from Reference^[23].

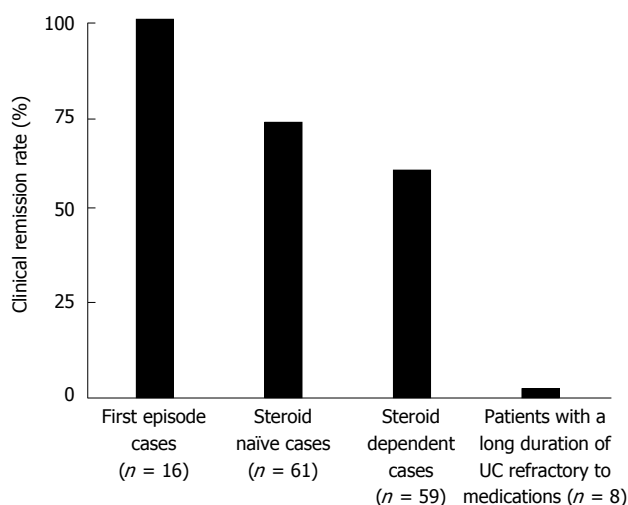


Figure 5 Clinical remission rates in typical cohorts of patients with active ulcerative colitis. The remission rate for patients with the first ulcerative colitis (UC) episode was 100%. All these first episode cases were steroid naïve with a short (< 4 mo) duration of UC; both steroid naïve and short disease duration are granulocyte and monocyte apheresis (GMA)-responder features. Accordingly, the remission rate in corticosteroid naïve cohort looks better than for steroid dependent cohort. GMA non-responder patients had deep ulcers with near total loss of the mucosal tissue at the lesion sites, a long duration of UC, and exposure to multiple drugs; a few were candidates for colectomy^[42,49,52].

GMA non-responder patients had deep ulcers with near total loss of the mucosal tissue at the lesion sites, a long duration of UC, and exposure to multiple drugs^[49,52]; a few were candidates for colectomy^[49,52]. Further, sustainabil-

ity of GMA induced remission has been good^[50,53,69,75,87]. Patients who relapse respond well to another course of GMA therapy^[87]. Regarding remission rates, Tanaka *et al*^[49] reported an efficacy of 73.8% based on CAI in a cohort of 61 steroid naïve patients. Similar efficacy rate has been reported by Hanai *et al*^[50].

GMA SPARES CORTICOSTEROID

In the first pivotal study by Shimoyama *et al*^[41] mentioned above, the steroid sparing effect of GMA was unequivocally demonstrated. In a multicentre setting^[22,41], 105 patients with active UC while on prednisolone (PSL) were randomly assigned to GMA ($n = 53$) or to PSL ($n = 52$). As seen on Figure 6, at entry, in the PSL group, the steroid dose was increased to induce remission, while patients in the GMA group received GMA, at one session per week over five consecutive weeks as remission induction therapy. As seen on the figure, PSL dose was then tapered in line with disease improvement or remission. At the end of the trial, 21 patients (44.2%) in the PSL group and 31 patients (58.5%) in the GMA group were in remission. The results of this study suggested that GMA has significantly better efficacy as compared with the conventional corticosteroid and can spare patients from steroids. Likewise, in a later study by Hanai *et al*^[44], the authors randomly assigned 69 patients with corticosteroid dependent moderately severe UC (at the time of relapse) to GMA ($n = 46$) or to PSL ($n = 23$).

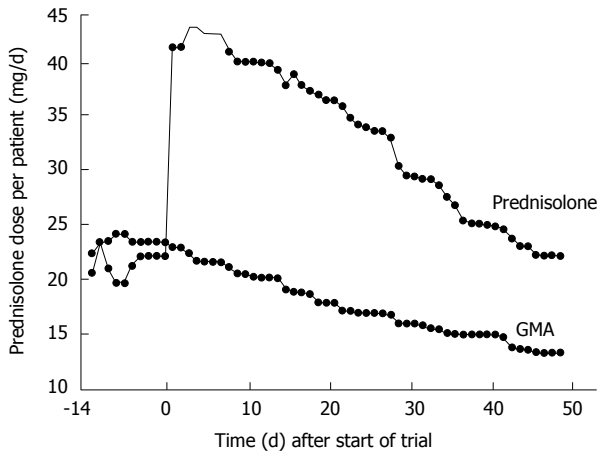


Figure 6 Steroid sparing effect of granulocyte and monocyte apheresis. In this trial, 105 patients with active ulcerative colitis while on the corticosteroid prednisolone (PSL) were randomly assigned to granulocyte and monocyte apheresis (GMA) ($n = 53$) or to PSL ($n = 52$). In the PSL group, the steroid dose was increased at entry to induce remission, while patients in the GMA group received GMA, at one session per week over five consecutive weeks. PSL dose was then tapered in line with disease improvement or remission. At the end of the trial, 21 patients (44.2%) in the PSL group and 31 patients (58.5%) in the GMA group were in remission. Therefore, GMA showed significantly better efficacy than PSL and spared patients from steroids^[22,41,44].

At entry, the mean dose of PSL was 12 mg per day per patient in both groups. As remission induction therapy, in the GMA group, patients were given up to an 11 treatment sessions over 10 wk, while in the PSL group, the mean dose of PSL was increased to 30 mg per day per patient. At week 12, an 83% of the patients in the GMA group were in clinical remission *vs* 65% of the patients in the PSL group. Further, during the 12 wk of treatment, the cumulative amount of PSL received per patient was 1157 mg in the GMA and 1938 mg in the PSL group. This study provided further support for GMA as an effective adjunct to standard drug therapy of moderately severe UC with significant corticosteroid sparing effect. They reported steroid related adverse side effects in a significant number of patients, while for GMA, side effects were flushing and transient lightheadedness in a small number of patients.

GMA FOR CHILDREN OR ADOLESCENTS WITH UC

In children and adolescents with IBD, conventional drugs like salicylates, thiopurines, corticosteroids or the new anti-TNF- α biologics may adversely affect the patients' growth and development. Therefore, GMA as a non-drug therapeutic intervention, if effective in this clinical setting is unrivalled by any currently known drug based medication. With this in mind, Tomomasa *et al.*^[76] were the first to apply GMA to children who had active UC. In that study, 12 children, age 5 to 15 years, all with active UC refractory to corticosteroids were given one GMA session per week for 5-10 consecutive weeks. In 8 patients, clinical symptoms improved after two GMA

sessions. Normal body temperature, stool frequency, and disappearance of blood in stool were seen after 24.3 ± 11.5 d. The endoscopic index improved from 2.6 ± 0.3 to 0.4 ± 0.2 . One patient who initially responded developed bloody diarrhoea later and 2 cases remained unchanged. The dose of steroid was tapered during GMA therapy by 50%. No serious adverse effect was observed. This study showed that GMA was effective and well tolerated in children with active UC refractory to corticosteroids.

Very recently, Tanaka *et al.*^[75] reported good efficacy and safety outcomes for GMA in children and adolescents with active IBD, all corticosteroid naïve, which is a GMA responder feature. In a single centre setting, a total of 24 consecutive children and adolescents, age 11-19 years were given mesalazine or sulphasalazine as a first-line medication. Seventeen patients relapsed or did not respond to the first-line medications, and received GMA at 2 sessions in the first week, and then weekly, up to an 11 sessions. Patients who achieved a decrease of at least 5 points in the CAI score were to continue with GMA, while non-responders were to receive 0.5 to 1.0 mg/kg per day PSL plus additional GMA sessions similar to GMA responder cases. At entry and week 12, patients were clinically and endoscopically evaluated, allowing each patient to serve as her or his own control. In this study, 7 patients achieved remission with the first-line medications and did not receive GMA. Five patients did not respond to the first 5 GMA sessions and received PSL plus GMA, while 12 patients responded to the first 5 GMA sessions and received additional sessions. At entry, the average CAI was 14.1 ± 0.4 , range 11-17, and the average endoscopic index was 9.2 ± 0.3 , range 7-11. The corresponding values at week 12 were 2.1 ± 0.2 , range 1-4 and 2.4 ± 0.2 , range 1-4. PSL was tapered to 0 mg within 3 mo in the 5 cases who did receive PSL in combination with GMA. This study is the closest one can expect to clinical practice rather than to clinical trial. With the strategy they applied, all 24 consecutive patients achieved clinical remission, most with endoscopic remission as well^[75]. The authors' conclusion states that in growing patients with active UC refractory to first-line medications, GMA induced clinical remission and mucosal healing, while in non-responders to GMA monotherapy, addition of a low dose PSL enhanced the efficacy of GMA and tapering of the PSL dose soon after remission was not associated with UC relapse. Avoiding corticosteroid at an early stage of UC should ensure better long-term clinical course^[70,75].

GMA IN PATIENTS WITH CD

So far, this review has focused mainly on the efficacy of GMA in patients with active UC. This is because IBD in the colon or in the rectum is strongly affected by neutrophils^[50,54], while the role of myeloid lineage leucocytes in the small intestinal IBD lesions has not been defined yet. Nonetheless the majority of CD patients have colonic lesions, and therefore, GMA seems to produce signifi-

cant efficacy in most patients with active CD^[85,97,98]. Additionally, as described for UC, patients with severe CD refractory to currently available pharmacologicals may not respond well to GMA^[93]. Perhaps most IBD physicians in the West are not fully aware that unlike Europe where there is no striking difference in the prevalence of UC and CD, in Japan, the prevalence of UC is more than 3 times higher than that of CD. Accordingly, the first Adacolumn multicenter study in Japan that led to reimbursement approval was in patients with active UC^[22,41]. This is why most GMA papers from Japan are on studies in patients with UC. However, subsequently, a clinical trial in patients with CD refractory to conventional medications was undertaken^[98], and led to reimbursement approval for patients with CD in Japan. However, the first study in CD was reported by Matsui *et al.*^[97], on 7 patients who were refractory to standard medications, each patient received 5 GMA sessions over 5 consecutive weeks. Five of 7 patients achieved remission. It is clinically relevant to mention here that the only 2 GMA non-responders in Matsui's study had primarily small intestinal lesions. Subsequently, Fukuda *et al.*^[98] reported an efficacy rate of 52% by applying 5 GMA sessions to each of 21 patients with severe CD. However, it is imperative to elaborate that the patients Fukuda *et al.*^[98] included had received conventional medications including 2 wk of optimum nutritional therapy (a routine treatment for CD in Japan) and only patients who remained with a high CDAI score received GMA. Therefore, 52% remission rate in a cohort of patients with severe and medication refractory CD was very encouraging. Further, Domènech *et al.*^[85], treated 12 steroid dependent patients with CD. The remission rate was 70%. Finally, Muratov *et al.*^[79] treated 7 patients with CD who had relapsed while on optimum conventional medication and a few on biologics. The median CDAI decreased from 290 at weeks 1 to 184 at week 7 and to 128.5 (remission level) at 12 mo. It is clear from the aforementioned reports that up to now, only very complicated and severe cases of CD have received GMA therapy.

EFFECTIVE DOSAGE OF GMA

The evolution of modern medicine has relied on the outcomes of clinical trials to determine the dosage of drugs with maximum efficacy margin and minimum adverse side effect. Fortunately for GMA, which is a non-drug treatment strategy, reliance on clinical trial outcomes has been less demanding or at least lack of it has not caused serious concern partly because of its good safety profile, and partly for the fact that GMA removes from the body instead of adding to it. Accordingly, unlike drugs, loss of efficacy, dependence and refractoriness are not likely. Nonetheless, it is a basic requirement to know the most effective frequency and the number of GMA sessions for patients with mild, moderate or severe IBD as this can help to save time and cost. The reality is that up to now GMA treatment has been an empirical practice. Some institutes administer 2 GMA sessions per week in the

first 2-3 wk and then 1 session per week up to 10 or 11 sessions^[50,52]. Hanai *et al.*^[50] reported that although patients with steroid naïve UC responded well to 5 GMA sessions, steroid refractory patients with severe UC responded better to 10 sessions. In contrast, Suzuki *et al.*^[42,52] administer 2 GMA sessions per week and cease when CAI decreases to 4 or less (clinical remission level); patients who do not improve after several sessions are classified as non-responders^[52]. These treatment regimens are all contrary to the initial clinical trials in which 5 GMA sessions over five consecutive weeks were applied^[22,41]. Regarding duration of one GMA session, Kanke *et al.*^[66] found that 90 min was significantly better than the routinely applied 60 min per GMA session. Likewise, Yoshimura *et al.*^[73] increased the processed blood volume from the conventional 1800 mL per GMA session to over 3000 mL per session. In this study, the efficacy rate in the higher processed blood volume group was significantly greater than in the 1800 mL per session group^[73]. In a prospective multicentre study, Sakuraba *et al.*^[99] found that intensive GMA at 2 sessions per week induced remission in shorter time and at a significantly higher rate when compared to weekly GMA. The authors assigned 112 patients with moderately active UC to 2 groups. Group 1 patients received one GMA session per week, while group 2 patients received 2 sessions per week, up to 10 sessions in both groups. The remission rate in group 1 was 46.7%, while in group 2 was 73.1%. Further, the mean time to remission was 28.1 d, in group 1 and 16.3 d in group 2. In spite of these outcomes, there is evidence to suggest that the efficacy of GMA is time dependent. Recently, Yamamoto *et al.*^[100] administered one GMA session per day over 5 consecutive days. There was no safety concern, but the efficacy rate was very much less than in the 5 GMA sessions reported by Shimoyama *et al.*^[41]. As reviewed above, in patients with active IBD, large numbers of myeloid lineage leucocytes in the colonic mucosa are seen in biopsies^[49,53] or by measuring a specific neutrophil protein^[101]. The infiltrated neutrophils may take several weeks to clear in spite of CAI showing clinical remission^[102]. Additionally, the immunomodulatory actions of GMA are time dependent. In line with this assertion, in rheumatoid arthritis patients, there was a sustained increase in CD4⁺ T-lymphocytes up to 12 wk following the last GMA session^[22]. Similarly, there was a striking down-modulation of the inflammatory chemokine receptor CXCR3 on leucocytes several weeks after the last GMA session^[23]. Clearly further investigations are warranted for establishing the optimum frequency and duration of GMA session.

TREATMENT COST

In a comprehensive study, Panés *et al.*^[78] estimated the treatment cost for patients with UC in the EU countries for conventional medications and GMA. The average annual cost per patient treated with conventional medications was estimated to be 6740€, while the cost for GMA was 6959€, which is very close to the conventional medi-

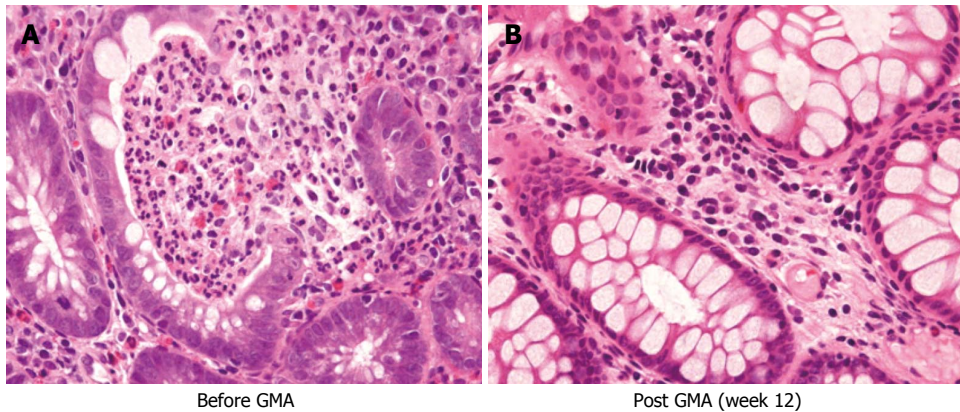


Figure 7 Typical immunohistochemical images taken from colonic biopsy specimens in a patient with total colitis in active stage (A) and following remission (B). This figure shows that the mucosal tissue is densely infiltrated by myeloid lineage leucocytes and granulocyte and monocyte apheresis has reduced the concentration of these leucocytes in the mucosa. The specimens seen in this figure are from a 60-year-old male with moderately severe ulcerative colitis and corticosteroid naïve, baseline clinical activity index, 13.

cations. In contrast, for steroid-dependent patients, the average annual cost was 6059€ for conventional medications and 11436€ for GMA. Further, this study found that the efficacy rate in patients who achieved clinical remission with GMA was 22.5% higher than for conventional therapy. The authors concluded that incorporating GMA in the therapeutic management of patients with moderate-to-severe UC, GMA is cost-effective and the extra cost is compromised by GMA's safety profile when compared with drug therapy.

GMA REDUCES MUCOSAL CONCENTRATION OF MYELOID LINEAGE LEUCOCYTES

As reviewed above, one pathologic feature of active IBD is presence of a large number of granulocytes and monocytes/macrophages in the colonic mucosa^[49,53]. In clinical settings, GMA is applied as an extracorporeal procedure to reduce the excess and activated myeloid lineage leucocytes from patients' systemic circulation. One could logically argue that removal of the leucocytes from the circulating blood should lead to depletion of these cells in the colonic mucosa where they are believed to exacerbate and perpetuate IBD^[25,27,36]. Figure 7 shows typical immunohistochemical images taken from colonic biopsy specimens in a patient with total colitis in active stage and following GMA induced remission. The figure shows that the colonic mucosal tissue is densely infiltrated by myeloid lineage leucocytes with the formation of crypt abscess and GMA has reduced the concentration of these leucocytes in the mucosa. It is relevant to mention here that the images seen in Figure 7 are typical for IBD lesions in the large intestine because similar images are uncommon in biopsies from small intestinal IBD lesions found in most patients with CD. It might be equally true to say that CD patients with the active IBD lesions confined to the small intestine may not be the right candidates for GMA therapy^[97].

COLONOSCOPIC FEATURES OF GMA RESPONDER AND NON-RESPONDER PATIENTS

As reviewed above, several studies have reported that any patient with a fair level of intact colonic mucosa is a potential responder to GMA. With this in mind, in the authors' hospitals, all patients receive endoscopic evaluations and the evaluation is used to treat or not to treat a patient by GMA. However, as stated above, by applying GMA at an early stage^[70], most first episode cases respond^[52], and we do not have many drug refractory patients with badly damaged mucosal tissue. Even patients with a near equal CAI score may have very different mucosal damage status, indicating that CAI per se does not reflect the full extent of mucosal damage in patients with IBD^[49]. Figure 8 shows typical colonoscopy features of patients who may respond well to GMA and be spared from drug based medications. These two cases were from a subgroup of patients who were identified as responders by colonoscopy. These cases were steroid naïve with severe UC based on clinical evaluation (CAI > 14) without endoscopy, nonetheless good responders to GMA because, firstly, the mucosal tissue was preserved and secondly, the patients were not exposed to multiple drugs prior to GMA. The photographs show complete restoration of the mucosal vascular patterns post GMA. Almost all such cases readily respond to GMA. Without colonoscopy, clinical evaluation would have identified these patients as having severe UC, a potential candidate for steroid therapy. However, such responders to GMA without corticosteroids should have good long-term clinical course^[70].

In contrast, Figure 9 shows colonoscopic features of GMA non-responder patients. Here, colonoscopy photographs from two typical GMA non-responder patients at entry and at week 12 are presented. The colonoscopy images at entry revealed deep and extensive colonic lesions with virtually no mucosal tissue left at the lesion sites.

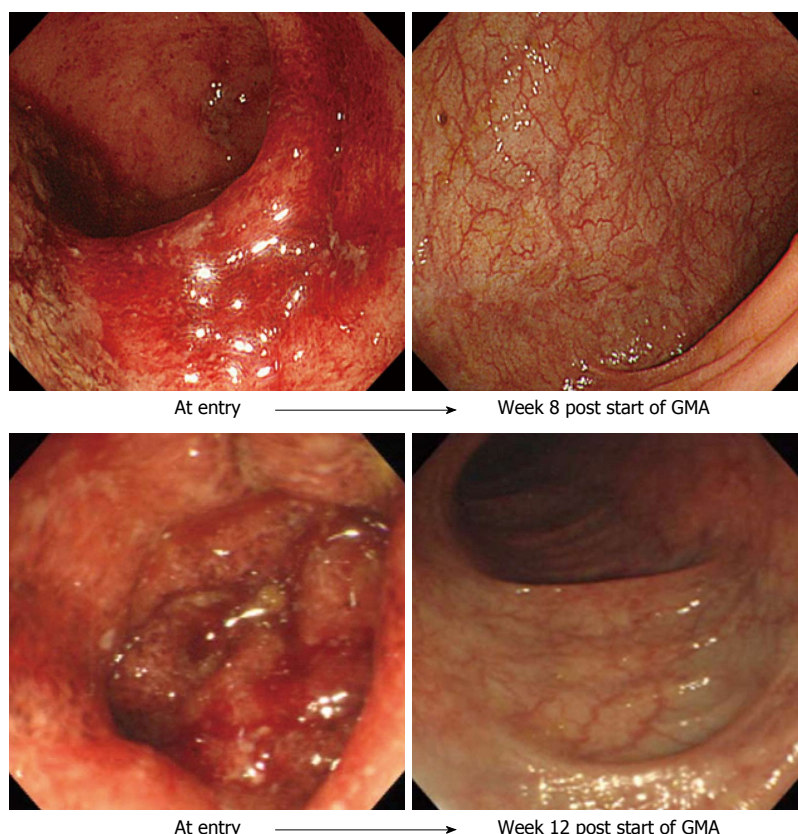


Figure 8 Typical endoscopic features of patients who may respond well to granulocyte and monocyte apheresis and be spared from drug based medications. These cases were from a subgroup of patients who have been identified as good responders by colonoscopy. These patients were steroid naïve with severe ulcerative colitis (clinical activity indices > 14), yet good responders to granulocyte and monocyte apheresis (GMA), firstly because the mucosal tissue was preserved and secondly, the patients were corticosteroid naïve at the time of relapse. The photographs show complete restoration of the mucosal vascular patterns at post GMA.

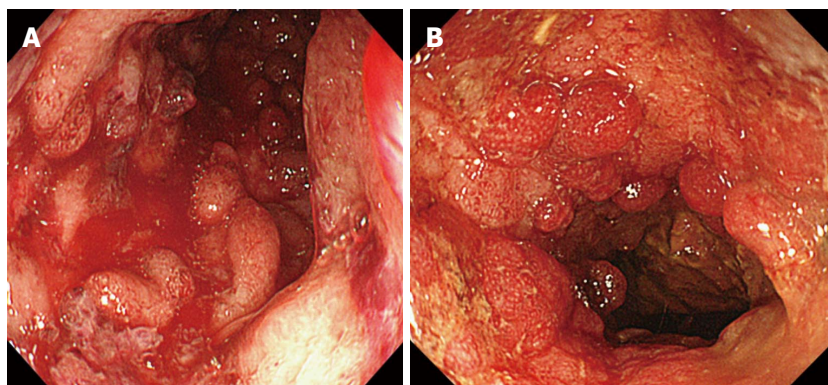


Figure 9 Colonoscopy images showing deep and extensive colonic lesions together with inflammatory polyps and contact bleeding. Typical colonoscopic images from patients with severely damaged mucosal tissue (A), granulocyte/monocyte apheresis non-responders (B). However, most patients with the entry mucosal damage seen in this figure are medication refractory and unlikely to respond to granulocyte and monocyte apheresis, some opt for colectomy.

However, no patient with the entry mucosal damage seen in this figure is likely to show any significant fall in CAI, a few may have fulminant UC, candidates for surgery.

TREATMENT OF PYODERMA

GANGRENOSUM ASSOCIATED WITH IBD

Pyoderma gangrenosum (PG)-like skin lesions are seen in up to 2% of patients with IBD and can be a major

cause of pathological and psychological morbidity^[103]. It is interesting that PG and psoriatic skin lesions are reported to be good responders to GMA^[103-107]. To our knowledge, most treated cases have responded well and yet, it all happened by chance^[103]. A patient with IBD associated PG was treated for IBD, but the response of pyoderma lesions was more striking than anything expected from GMA. The young patient had very stressful deep refractory lesions over a long period of time (Figure 10). The lesions fully remitted after 10 GMA sessions^[103].

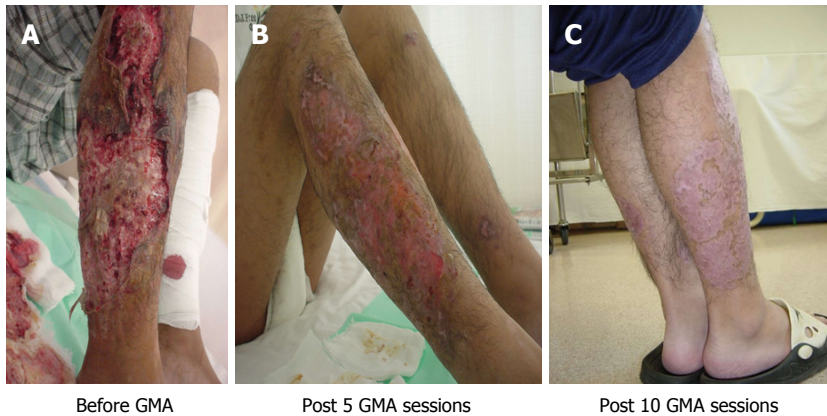


Figure 10 Pyoderma gangrenosum lesions. Pyoderma gangrenosum lesions associated with Crohn's disease (A), partially re-epithelialized after 5 granulocyte and monocyte apheresis (GMA), sessions (B), fully re-epithelialized after 10 GMA sessions (C)^[103].

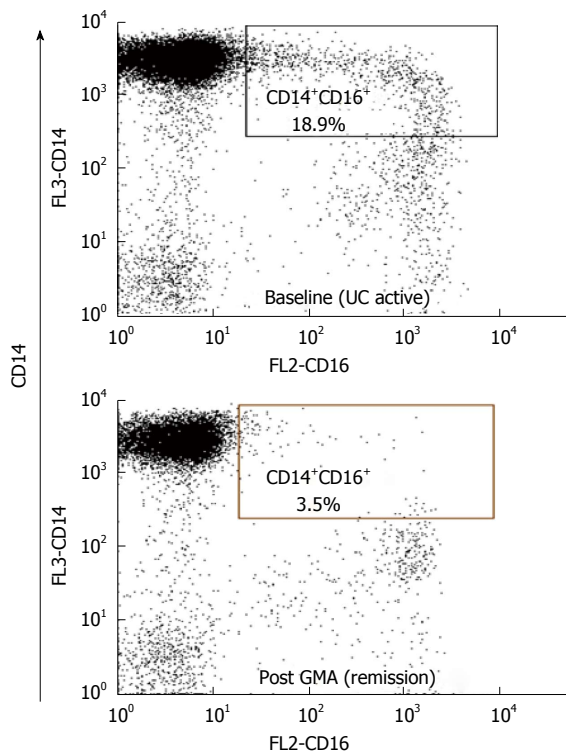


Figure 11 Typical flow cytometry outputs. Flow cytometry showing immunophenotyping of elevated pro-inflammatory CD14(+)CD16(+)DR(++) monocytes in a patient with inflammatory bowel disease and depletion of these tumour necrosis factor-producing leucocytes by granulocyte and monocyte apheresis (GMA). In this patient, an 18.9% of monocytes were identified as CD14⁺CD16⁺ phenotype in active ulcerative colitis (UC) stage and this was reduced to just 3.5% when the patient achieved remission following a course of GMA therapy. Normal level is < 8%^[109].

Subsequently, other authors independently reported cases of pyoderma or psoriatic skin lesions responding well to GMA^[104-107]. The mechanism associated with the efficacy of GMA in these conditions is not very clear, but the chemokine receptor, CXCR3 is known to have an active role in the initiation and perpetuation of inflammatory skin lesions and is strongly down-modulated by GMA^[23]. This action of GMA, if duplicated by follow-up studies can serve as a major break-through in our efforts to understand the mechanisms of clinical efficacy of GMA in

these clinical settings.

SPECIFIC IMMUNOLOGICAL EFFECTS

The CD14⁺CD16⁺ monocyte phenotype is known to be pro-inflammatory as a major source of TNF^[108] and shows dramatic expansion in inflammatory conditions. Accordingly, Hanai *et al*^[109] reported elevated CD14⁺CD16⁺ monocytes in patients with IBD and a very marked depletion of these TNF-producing monocytes by GMA. This monocyte sub-set was depleted to levels seen in healthy persons^[109]. In Figure 11, typical effect of GMA is shown by flow cytometry. This action of GMA should significantly alleviate inflammation in patients with dysregulated immune profile. Additionally, as reviewed above, GMA has been associated with a sustained increase in circulating lymphocytes^[20] including the CD4(+)CD25(+) Treg phenotype^[61]. In spite of this, the clinical efficacy associated with a course of GMA is unlikely to reflect its effects on peripheral leucocytes per se. It is inferred that additional mechanisms of actions might follow a course of GMA. As seen in Figure 4, leucocytes that bear the FcγR and complement receptors adhere to the GMA carriers^[23,62]. The adsorbed leucocytes release an array of substances both toxic and non-toxic, but anti-inflammatory as well, which reach the patients' circulation *via* the column outflow that returns to patients. Among these, cytokines, C3a and C5a are of short half-life and may not reach the patients' circulation in fully active form. Hanai *et al*^[110] reported a significant increase in the column outflow blood levels of soluble TNF receptors I and II known to neutralize TNF without invoking TNF-like actions^[111].

Further, GMA-related suppression of inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) released by peripheral leucocytes has been reported^[112]. In in-vitro settings, exposure of human blood to the GMA carriers caused the release of significant amounts of IL-1 receptor antagonist (IL-1ra) and hepatocyte growth factor (HGF)^[113], seen in Figure 12. The release of these substances (albeit very small) should proceed inside the Adacolumn during GMA in clinical settings and reach the patients' circulation *via* the column outflow line. IL-

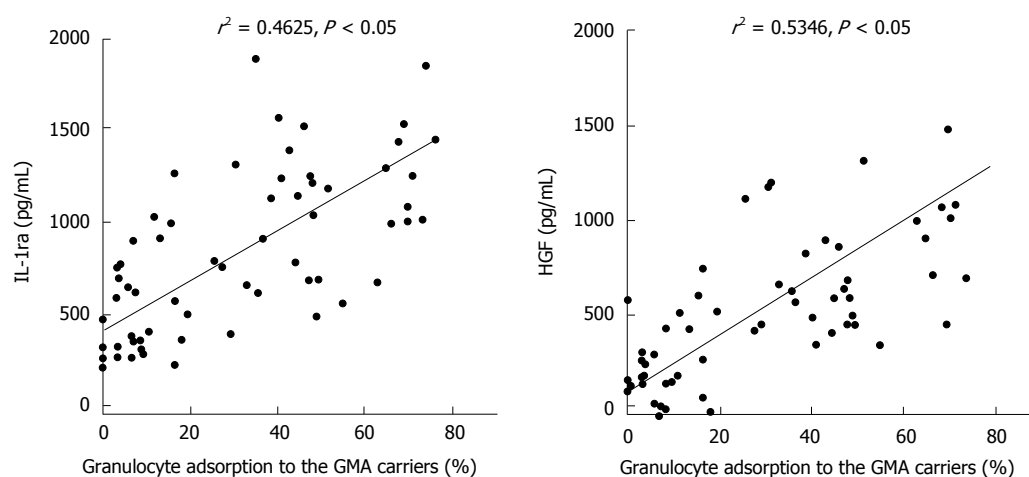


Figure 12 Adsorption dependent release of interleukin-1 receptor antagonist, and hepatocyte growth factor from myeloid lineage leucocytes (granulocytes and monocytes) in *in-vitro* setting. Hepatocyte growth factor (HGF) is known to promote ulcer healing and cell regeneration, while interleukin-1 receptor antagonist (IL-1ra) is strongly anti-inflammatory^[113]. GMA: Granulocyte and monocyte apheresis.

1ra is known to control intestinal inflammation^[114], while HGF is believed to promote epithelial cell regeneration, an essential step in ulcer healing^[115]. Further, examinations of mucosal biopsy specimens suggested that GMA suppresses cytokine profiles within the mucosa^[79,116]. A wary reader may wonder why this is possible with a system that impacts peripheral blood leucocyte counts. One answer could be that GMA reduces the number of leucocytes that are destined for the mucosa, partially by depleting them (Figure 3) and partially by down-modulating the adhesion receptors on the remaining leucocytes^[117]. Muratov *et al.*^[79] found a marked decrease in tissue interferon (IFN)- γ or TNF- α positive leucocytes in clinical responders after GMA. In parallel, significantly lower levels of IFN- γ producing leucocytes were detected in peripheral blood. IFN- γ positive T-cells in pretreatment biopsies completely disappeared or decreased in post-treatment biopsies sampled 2 wk after the last GMA session in responders and appeared to predict the maintenance of long-term remission, up to 12 mo. However, our view is that the impact of GMA on the CD4(+)CD25(+) Treg^[61] reviewed above is potentially very interesting in patients with dysregulated immune behaviour in whom, the immune system is in a state of exuberant activity. In Figure 13, the immunological actions of GMA in patients with IBD are summarized based on published findings. In addition to adsorption of elevated and activated myeloid lineage leucocytes to the GMA carriers, the column outflow blood is a potential source of anti-inflammatory substances.

PUTTING EVERYTHING TOGETHER

IBD is a debilitating chronic, life-long health disorder, affecting millions of individuals with symptoms, which impair function and quality of life. The aetiology of IBD is not precisely understood at present, and therefore, hitherto drug therapy has been empirical rather than based on a sound understanding of disease mechanism(s). This empirical approach to medication might be a major fac-

tor for refractoriness and adverse drug effects. Indeed, adverse drug side effects become additional morbidity factors in many patients on long-term medications. Although, the fundamental cause(s) of IBD is not well known, but the exacerbating factors like TNF- α and other inflammatory cytokines are known and have been targeted. Bearing in mind that inflammatory cytokines are generated by the patients' own cellular elements including myeloid lineage leucocytes, it seems logical to apply GMA and deplete the elevated and activated myeloid lineage leucocytes. When the elevated cytokine producing leucocytes are depleted, the patients' immune system should be able to adjust itself to a more normal function or at least drug efficacy could be better. As GMA removes from the body instead of adding, it has not been associated with dependency, refractoriness, and safety concern. However, let's not forget that efficacy outcomes with GMA in patients with IBD have been impressive as well as disappointing. A review of the clinical experience indicates that GMA responders and non-responders define patients' past disease course, severity and response to medications. This is to say that IBD patients present with diverse clinical and endoscopic disease severity levels, long or short duration of IBD, and a history of exposure to medication (or otherwise). Hence, their clinical response to medical interventions or to GMA can be complete remission, partial response or no response at all. Regarding GMA, it has been found that first episode cases together with steroid naïve and patients with a short duration of active IBD, but without extensive loss of the mucosal tissue respond well and are spared from additional pharmacologic interventions. Additionally, GMA responder patients have good long-term disease course. Therefore, if this strategy is adhered to at an early stage during the development of IBD, there should be fewer drug-refractory or cases with badly damaged mucosal tissue that are medication or GMA non-responders. GMA is not a rescue therapy in patients in whom drug therapy has failed, while disease severity is worsening, but rather

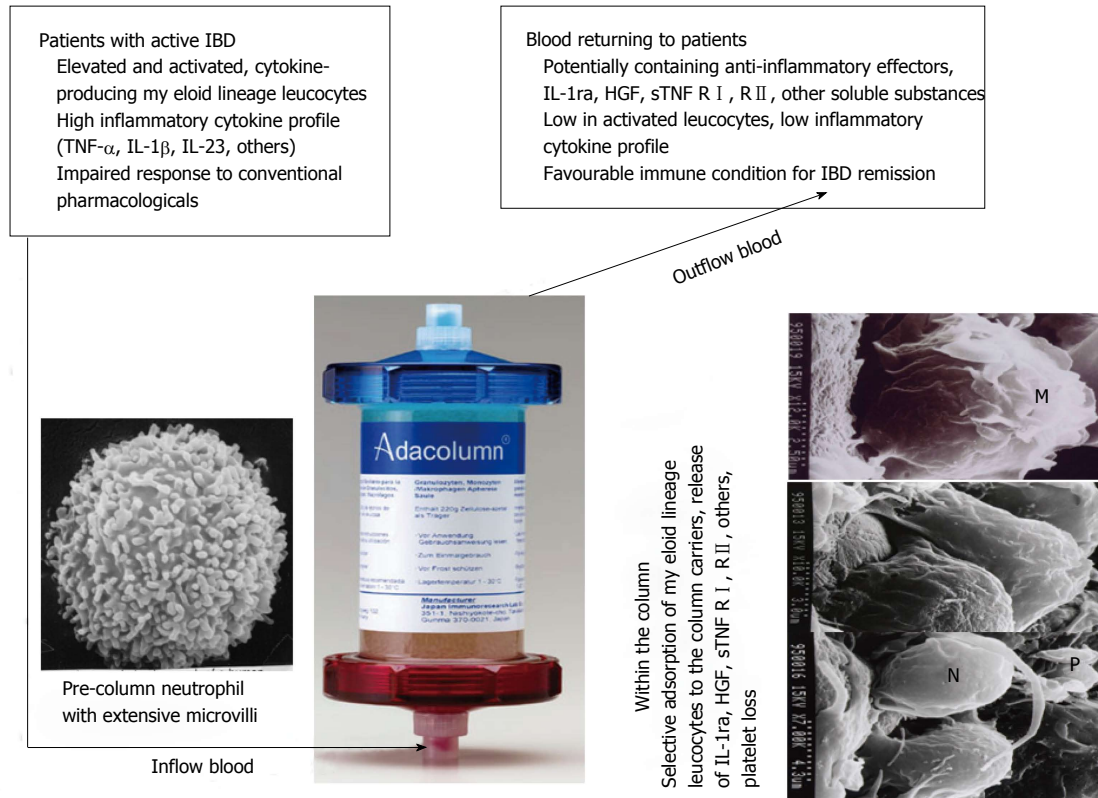


Figure 13 An idealistic view of the events attributed to therapeutic granulocyte and monocyte apheresis with the Adacolumn in patients with inflammatory bowel disease. Scanning photomicrographs of leucocytes adsorbed onto a granulocyte and monocyte apheresis (GMA) carrier and the basic events, which follow on the surface of the carriers during GMA in clinical setting (see Figure 4 as well). Normal, non-activated and non-adherent neutrophils and monocytes/macrophages express extensive surface microvilli. The villi bear various receptors like tumour necrosis factor (TNF) receptors, and L selectin, which are shed upon adsorption of the cells to the carriers. Further as seen, adsorbed leucocytes undergo extensive release reaction. Up to now, interleukin-1 receptor antagonist (IL-1ra), hepatocyte growth factor (HGF), IL-10 and soluble TNF receptors have been measured. During GMA, the blood, which returns to patients via the column outflow line may be likened to a biologic cocktail containing a large number of soluble substances released by the adherent leucocytes. The adsorptive nature of GMA is thought to be the most intriguing feature of this non-pharmacological treatment intervention. M: Monocyte; N: Neutrophil; P: Platelet; IBD: Inflammatory bowel disease.

to minimize the number of patients who need such intervention. Centuries ago, bloodletting was a major medical practice to cure disease because of lack of today's medicines, while today, GMA therapy reflects advance in our knowledge of immune behaviour in IBD and a desire to treat without drugs.

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WJG 20th Anniversary Special Issues (5): Colorectal cancer

Colorectal cancer in inflammatory bowel disease: The risk, pathogenesis, prevention and diagnosis

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Abstract

Patients with inflammatory bowel disease (IBD) are at increased risk for developing colorectal cancer (CRC), although the overall incidence of IBD-associated CRC has been diminishing in recent decades in western countries. As demonstrated in previous studies, the risk of CRC in IBD increases with longer duration, extent of colitis, a familial history of CRC, coexistent primary sclerosing cholangitis, and the degree of inflammation. The pathogenesis of CRC in IBD is poorly understood. Similar to sporadic CRC, IBD-associated CRC is a consequence of sequential episodes of genomic alteration. Multiple inter-related pathways, including immune response by mucosal inflammatory mediators, oxidative stress, and intestinal microbiota, are also involved the pathogenesis of IBD-associated CRC. Continuing colonic inflammation appears to be a factor in the development of CRC; therefore, anti-inflammatory agents such as 5-aminosalicylate compounds and immune modulators have been considered as potential chemopreventive agents. Colonoscopic surveillance is widely accepted as being effective in reducing the risk of IBD-associated CRC, although no clear evidence has confirmed that

surveillance colonoscopy prolongs survival in patients with extensive colitis. The traditional recommendation has been quadrantic random biopsies throughout the entire colon; however, several guidelines now have endorsed chromoendoscopy with a target biopsy because of increasing diagnostic yields and reduced workloads for endoscopists and pathologists. New technologies such as narrow band imaging, confocal endomicroscopy, and autofluorescence imaging have not yet been confirmed as surveillance strategies in IBD.

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Key words: Inflammatory bowel disease; Colorectal cancer; Pathogenesis; Chemoprevention; Surveillance

Core tip: An updated comprehensive review on the risk, pathogenesis, prevention and diagnosis of colorectal cancer in inflammatory bowel disease.

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INTRODUCTION

Inflammatory bowel disease (IBD) is widely accepted as one of the important risk factors leading to colorectal cancer (CRC). IBD ranks as the third highest risk condition for CRC, behind only familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer syndrome (HNPCC)^[1]. In fact, CRC accounts for one sixth of ulcerative colitis-related deaths^[2].

Recently, a large nationwide Japanese study reported poorer survival for patients with ulcerative colitis (UC)-

Table 1 Summary of cumulative risk of colorectal cancer in patients with inflammatory bowel disease in recent population-based studies and meta-analysis

Study	Country (n)	Population (n)	Observed period	Incidence of colorectal cancer
Eaden <i>et al</i> ^[5]	United States (4), United Kingdom (7), Scandinavia (3), other (5)	UC (meta-analysis of 19 studies)	1925-1999	Cumulative incidence 1.6% by 10 yr of disease 8.3% by 20 yr of disease 18.4% by 30 yr of disease
Winther <i>et al</i> ^[6]	Copenhagen County, Denmark	1161 UC	1962-1987	Cumulative probability 0.4% by 10 yr of disease 1.1% by 20 yr of disease 2.1% by 30 yr of disease
Lakatos <i>et al</i> ^[14]	Veszprem, Hungary	723 UC	1974-2004	Cumulative risk 0.6% by 10 yr of disease 5.4% by 20 yr of disease 7.5% by 30 yr of disease
Rutter <i>et al</i> ^[84]	London, United Kingdom	600 long standing extensive UC	1971-2000	Cumulative incidence 0% by 10 yr of disease 2.5% by 20 yr of disease 7.6% by 30 yr of disease 10.8% by 40 yr of disease
Söderlund <i>et al</i> ^[8]	Sweden	7607 UC	1954-1989	Cumulative risk 1.5% by 10 yr of disease 3.8% by 20 yr of disease 7.6% by 30 yr of disease
Canavan <i>et al</i> ^[9]	United States (3), Denmark (3), United Kingdom (2), Sweden (2), Canada (1), Israel (1)	11545 CD (meta-analysis of 12 studies)	1972-2004	Cumulative risk 2.9% by 10 yr of disease 5.6% by 20 yr of disease 8.3% by 30 yr of disease
Kim <i>et al</i> ^[7]	South Korea	7061 UC	1970-2005	Cumulative risk 0.7% by 10 yr of disease 7.9% by 20 yr of disease 33.2% by 30 yr of disease

CD: Crohn's disease; UC: Ulcerative colitis.

associated CRC than with sporadic CRC in the advanced stage. In addition, UC-associated CRC tended to be of higher histologic grade, such as mucinous or signet ring cell type, and had a greater multiplicity in number when compared to sporadic CRC^[3]. This finding is similar to results reported in the large-scale nationwide population-based Danish study^[4], where the overall mortality rate ratio for UC-associated CRC patients compared with sporadic CRC patients was 1.24 (95%CI: 1.02-1.51) in the first year and 1.17 (95%CI: 1.01-1.36) after 5 years of follow-up. Therefore, identifying the at-risk patients and carrying out appropriate preventions such as chemoprevention or surveillance for these patients is of great importance in managing the CRC risk in IBD.

This review discusses the established risk factors, pathogenesis, and prevention and diagnosis of colon carcinogenesis in patients with IBD.

RISK FACTORS

The most important and well-recognized risk factors identified for IBD-associated CRC are disease duration and extent. The meta-analysis by Eaden *et al*^[5], which included 116 studies from a wide array of centers and geographic sites, was one of several milestones in establishing the incidence and cumulative risk of UC-associated CRC. This global meta-analysis reported that the

incidence, which measures risk over time, was 0.3% per year, or 3 cases of CRC per 1000 patient-years of follow up. Based on long term follow up in a subset of studies included in the meta-analysis, the cumulative risk of CRC in patients with left-sided disease or pancolitis was estimated at 1.6% at 10 years, 8.3% at 20 years, and 18.4% at 30 years after the development of UC. However, the more recently published population studies demonstrated contradictory results (Table 1). A Danish population-based cohort study reported that the cumulative probability of CRC was 0.4% at 10 years, 1.1% at 20 years, and 2.1% at 30 years after the development of UC^[6]. However, these results must be interpreted with caution because they reported high rates of proctocolectomy. Apart from this, several large studies from Hungary, the United Kingdom, and Sweden reported the cumulative risk of CRC in UC as 0.6%-1.5% at 10 years, 2.5%-5.4% at 20 years, and 7.5%-10.8% at 30 years; these studies suggest a low incidence rate of CRC. Recently, a nationwide Korean study involving 7061 patients with UC reported an estimated cumulative risk of ulcerative colitis-associated CRC of 0.7% at 10 years, 7.9% at 20 years, and 33.2% at 30 years^[7]. These results were comparable to Eaden's meta-analysis^[5].

This discrepancy between Western and Asian studies is thought to arise for several reasons. The incidence of ulcerative colitis seems to have reached a plateau in

Western countries; thus, colitic cancer development may also be at a steady state or else decreasing^[8]. However, in Asian countries, ulcerative colitis is still increasing; therefore, the occurrence of colitis cancer is also anticipated to increase. A meta-analysis of 12 published articles by Canavan *et al.*^[9] reported that the cumulative risk for patients with Crohn's disease (CD) was 2.9% at 10 years, 5.6% at 20 years, and 8.3% at 30 years after the CD diagnosis. This study showed that cumulative risk of developing CRC, once diagnosed with CD, is comparable to the risk associated with UC^[9].

The extent of colitis is an important risk factor, along with duration, for the development of CRC. The meta-analysis by Eaden *et al.*^[5] reported that an overall prevalence of 3.7% for CRC among patients with UC in all 116 studies, but when restricted to the 35 studies that stratified their analyses by extent of UC, the prevalence of CRC among patients with extensive involvement rose to 5.4%^[5,10]. A recent Danish population-based cohort study involving 1515 patients diagnosed with UC also reported that the risk of CRC in patients diagnosed with UC was highest among patients with extensive colitis (standardized incidence ratio, 1.85; 95%CI: 0.60-4.32). Several studies have shown a general consensus indicating little or no increased risk of CRC in patients with proctitis or proctosigmoiditis, whereas the risk is intermediate in those with left-sided colitis and highest with pan-colitis^[5,7,8,11-14].

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease that is strongly associated with IBD. An increased risk of CRC has been observed in patients with IBD complicated by PSC. A meta-analysis by Molodecky *et al.*^[15] reported that the pooled proportion of IBD in PSC cases was 68% (58%-77%). Among IBD subtypes, PSC was more common in UC than CD (80% *vs* 10% of cases)^[16]. The association with IBD was stronger with more extensive colonic involvement: in patients with pancolitis, the prevalence of PSC was about 6%, in contrast to 1% in those with only distal colitis^[17]. A meta-analysis of 11 studies involving 16844 patients with UC by Soetikno *et al.*^[18] reported that, overall, 21% of the patients with both UC and PSC developed colorectal neoplasms, compared with 4% of patients without PSC. The OR of developing dysplasia or cancer in patients with PSC was thus 4.8 (95%CI: 3.6-6.4), and this increased risk was present even when the risk of CRC alone was considered (OR = 4.3; 95%CI: 2.8-6.5).

Familial history of sporadic CRC increases the risk of CRC by at least two-fold when compared to patients with IBD without familial history for CRC^[19,20]. A population-based cohort study of patients with IBD reported that a family history of CRC was associated with a more than 2-fold risk of IBD-associated CRC (adjusted RR = 2.5; 95%CI: 1.4-4.4); furthermore, patients with a 1st-degree relative diagnosed with CRC before 50 years of age also had a higher risk (RR = 9.2; 95%CI: 3.7-23)^[20].

Chronic inflammation is believed to promote carcinogenesis^[21]. Several lines of evidence indicate that chronic inflammation is a key risk factor for CRC in patients with

IBD^[22]. However, few studies have directly investigated whether the level of inflammation correlates with CRC risk in patients with IBD^[21]. A retrospective case-control study^[23] reported a significant correlation between the colonoscopic and histologic inflammation scores and the risk of colorectal neoplasia. However, multivariate analysis revealed that only the histologic inflammation score remained a significant risk factor. Another retrospective cohort study of 418 patients undergoing colonoscopic surveillance for UC showed similar results, where each unit increase in inflammation score conferred a 3.8-fold increase in the risk for high-grade dysplasia or CRC over time, based on use of a 4-point scale to classify histologic inflammation from a previous retrospective case control study^[23,24]. On the other hand, Velayos *et al.*^[25] reported that the history of pseudopolyps increased the risk of CRC in UC by 2.5-fold (95%CI: 1.4-4.6), as a marker of more severe inflammation in the past.

PATHOGENESIS

The pathogenesis of CRC in IBD is poorly understood. Both genetic and environmental factors contribute to this pathogenesis, and the factors responsible for neoplastic changes in IBD can be summarized as follows: genetic instability; epigenetic alteration; immune response by mucosal inflammatory mediators; oxidative stress; and intestinal microbiota^[21,26,27].

Many of the genetic alterations associated with development of sporadic CRC also play roles in colitis-associated CRC^[27,28]. Colon carcinogenesis in IBD is thought to be similar to the adenoma-carcinoma sequence found in sporadic CRC. However, unlike sporadic CRC, which develops from dysplasia in 1 or 2 foci of the colon, cancer arising in colitis mucosa usually develops from multifocal dysplasia, indicating a "field change effect"^[1,21]. Aneuploidy, a marker of genomic instability, is demonstrated at 20%-50% in dysplastic lesion and 50%-90% of cancers and is detected in long-standing UC^[1,27,29,30]. Because aneuploidy is often more widespread than dysplasia in IBD, substantial genomic alterations must occur in colonic mucosa without disturbing morphology^[1,21].

The two major types of genomic instability found in CRCs are chromosomal instability (CIN) and microsatellite instability (MSI)^[31]. CIN and MSI in colitis-associated CRC appeared with the same frequency (85% CIN, 15% MSI) as seen in sporadic CRC, but they differed in the timing and frequency from the pattern seen with sporadic CRC (Figure 1)^[10,21,27,32]. For example, loss of adenomatous polyposis coli (*APC*) function, considered to be a very common initiating event in sporadic colorectal carcinogenesis, is less frequent and usually occurs later in the colitis-associated dysplasia-carcinoma sequence^[32-34]. Loss of *P53* function is an important step in the progression of colitis-associated cancer. In contrast to sporadic CRC, *P53* mutation in patients with IBD occurs early and is often detected in mucosa that is nondysplastic^[26,27,35]. MSI, due to defective DNA mismatch repair, has been

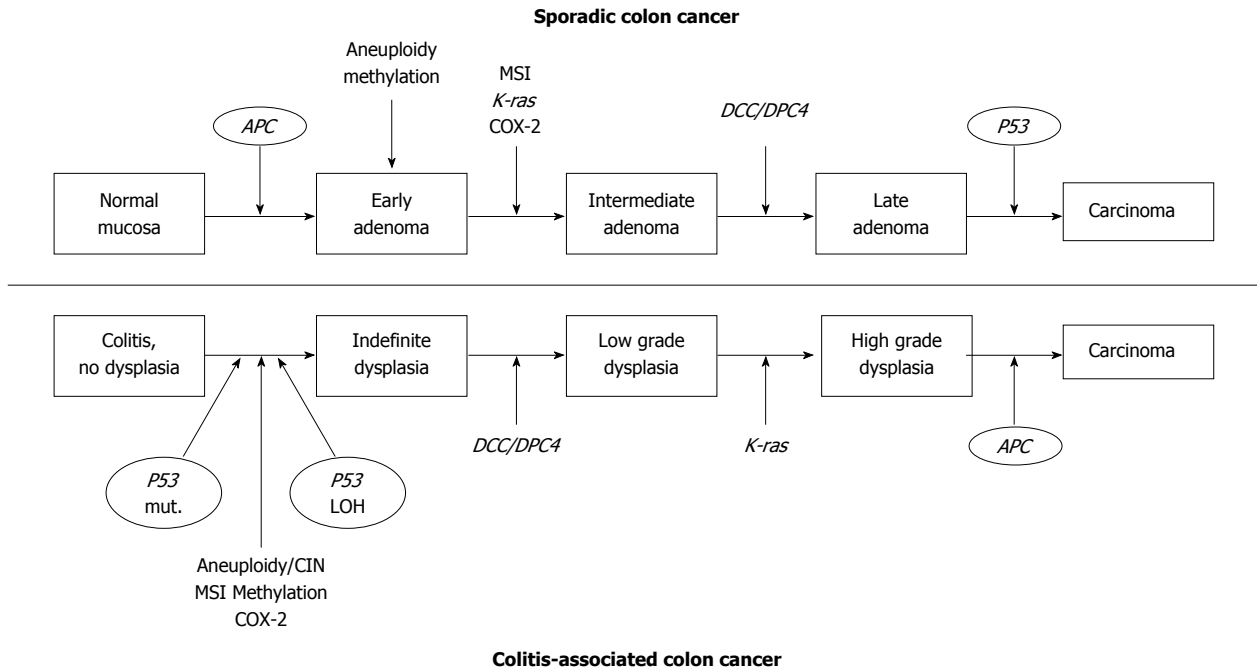


Figure 1 Molecular pathogenesis of sporadic colon cancer and colon cancer associated with inflammatory bowel disease. Many of the genetic alterations associated with development of sporadic colon cancer also play a role in colitis associated colon cancer. However, the frequency and sequence of these events differ between the cancers. Modified from Ref [21]. APC: Adenomatous polyposis coli; MSI: Microsatellite instability; KAS: Kirsten Rat Sarcoma Viral Oncogene Homolog; COX-2: Cyclooxygenase-2; DCC: Deleted in colorectal carcinoma; DPC4: Deleted in pancreatic carcinoma 4; CIN: Chromosomal instability.

reported to occur at variable frequencies in IBD associated CRC^[28,36-38]. The MSI-high (MSI-H) phenotype is observed in approximately 10% to 15% of sporadic CRC^[36].

The CpG island hypermethylation is a key epigenetic mechanism of gene silencing of tumor suppressor genes, including certain DNA repair genes where it acts through hypermethylation of their promoters^[28,32]. Promoter hypermethylation of the mismatch repair gene *hMLH1* is strongly associated with MSI. Among neoplastic samples from patients with colitis, *hMLH1* hypermethylation was observed in 6 of 13 (46%) patients with high levels of MSI, 1 of 6 (16%) with low levels of MSI, and 4 of 27 (15%) without MSI, so it might cause MSI or contribute to its development^[28,32,38]. Likewise, p16INK4a, a cell cycle inhibitor that is also implicated in sporadic colon cancer, is commonly hypermethylated in UC tissues, even in the absence of dysplasia^[28,39].

Other key elements in the pathogenesis of CRC in IBD are related to chronic inflammation, such as the induction cyclooxygenase (COX)-2, inflammatory cytokines, and chemokines. Some evidence indicates that NSAIDs decrease the risk of CRC in IBD patients by 40%-50%^[26,40]. NSAIDs exert their effects through their action on COX enzymes. Among three isoforms of COX enzyme, COX-2 is induced by inflammation and triggered by inflammatory stimuli such as IL-1, IFN- γ , and TNF- α ^[26]. Previous studies have shown that COX-2 expression is elevated in nearly 50% of adenomas and 85% of adenocarcinomas^[41,42]. Agoff *et al*^[43] reported that COX-2 messenger RNA and COX-2 protein over-expression occurred early in UC-associated neoplasia.

TNF- α is released by activated macrophages and T cells; it binds to the receptor TNF-receptor (TNF-R) and has been reported to promote inflammation and colitis-associated cancer^[21]. Several studies have assessed the relationship between the polymorphism of TNF- α -308 G>A and susceptibility to IBD and CRC^[26]. Recently, a meta-analysis study suggested that the polymorphism of TNF- α -308 G>A participates in modifying the susceptibility to UC and CD in Europeans and Asians^[44]. However, the increased risk of CRC in IBD patients should be clarified as the combined effects of polymorphisms in TNF- α and other cytokines and the interaction with environmental factors^[44].

Oxidative stress also contributes to pathogenesis of the colon carcinogenesis by attacking proteins and nucleic acids, resulting in denaturation and a variety of alterations including base modifications, double-base lesions, and strand breaks^[21,45,46]. Oxidative stress develops in response to inflammatory reactions in particular because the inflammatory cells, activated neutrophils, and macrophages produce large amounts of reactive oxygen and nitrogen species (RONS)^[21,26,28]. Inflamed tissues from patients with active UC or CD demonstrate increased expression of nitric oxide synthase (NOS) and RONS^[47-49]. RONS can interact with key genes involved in carcinogenic pathways such as *P53* and DNA mismatch repair genes^[28]. A previous study showed higher levels of *P53* mutation frequencies of both G:C to A:T transitions at the CpG site of codon 248 and C:G to T:A transitions at codon 247 in the inflamed tissue than in the noninflamed tissue of the colon, as well as in colon tissue from normal adult con-

trols^[47]. Another study reported that noncytotoxic levels of hydrogen peroxide dramatically reduced the activities of the mismatch repair system^[45].

Although intestinal microbiota have been considered as contributors to development of colorectal neoplasia in IBD, the mechanism of intestinal microbiota-induced carcinogenesis remains largely unclear^[26,27,50]. Several different rodent models of IBD have suggested that commensal or specific bacteria contribute to the development of colitis-associated cancer. For example, the IL-10 knock-out mice showed IBD (primarily colon and rectum) induced by *Enterococcus faecalis*, as well as rectal dysplasia and adenocarcinoma^[51]. Under specific pathogen free conditions, all IL-10 gene knock-out mice also developed colitis after 3 mo of age, and CRC observed in 25% and 60% of the mice after 3 and 6 mo, respectively^[52]. Modification of enteric flora in IL-10 knockout mice by probiotic lactobacilli was associated with a reduced prevalence of colon cancer and mucosal inflammatory activity^[53].

CHEMOPREVENTION

Chemoprevention has been shown to reduce the risk of adenoma and CRC in the general population without IBD^[54]. Pinczowski *et al.*^[55] first reported a chemopreventive effect of sulfasalazine in IBD, and several agents with chemopreventive potential have since been identified, including 5-aminosalicylate (5-ASA) compounds, immunomodulators, ursodeoxycholic acid (UDCA), and folic acid^[56]. However, prospective randomized controlled trials on chemoprevention are lacking in patients with IBD because of insurmountable obstacles such as ethical problems; consequently, data on chemoprevention in patients with IBD are not clearly definitive and refer to either retrospective case-control or cohort studies^[27,56].

Chronic inflammation has been accepted as one of the causative factors of IBD-associated CRC. The 5-ASA compounds are used as maintenance therapy in patients with UC because they have been shown to reduce inflammation in patients with UC. A meta-analysis of nine studies (three cohort and six case-control studies) that included 1932 UC patients reported that use of 5-ASA in UC was associated with a reduced risk of CRC, and the benefit occurred with regular use and use of at least 1.2 g of mesalamine equivalents per day^[57]. However, subsequent to this meta-analysis, more studies reporting negative results have emerged^[58,60]. A population based case control study showed that among patients with IBD (364 CRC cases, 1172 controls), exposure to 5-ASA therapy of any dose or duration during the 12 mo before CRC diagnosis was not associated with a reduced risk of CRC (OR = 0.97; 95%CI: 0.77-1.23)^[58]. Recently, Bernstein *et al.*^[59] used the Manitoba IBD epidemiology database to show that 5-ASA was not chemoprophylactic in IBD for CRC in either a population-based case control or a cohort analysis. Although the chemopreventive role of 5-ASA compounds has biological plausibility, the chemopreventive effects of 5-ASA in IBD remain conflicting.

Immunomodulators also have anti-inflammatory properties and are used as maintenance therapy in IBD patients. Only limited evidence exists for a chemopreventive role for immunomodulators in IBD. A recent population-based study using a database linked to a nationwide pathology network from the Netherlands demonstrated a significant chemopreventive effect of thiopurine in patients with IBD (the adjusted hazard ratio: 0.10; 95%CI: 0.01-0.75). However, the majority of studies have not shown a chemopreventive effect for immunomodulators^[25,58,61,62]. An 8-year follow up study reported no significant differences in rates of progression to advanced neoplasia or to any neoplasia between azathiopurine/6-MP user and never-user patients with UC^[62]. Another retrospective cohort study also failed to demonstrate any preventive effect of azathiopurine/6-MP^[24].

The chemopreventive role of UDCA therapy is well-supported from studies of patients with concomitant with IBD and PSC. The colonic concentration of additional bile acids has been implicated as a carcinogen, as this is cytotoxic to colonic epithelial cells and induces hyperproliferation^[56]. Therefore, studies have suggested that treatment with UDCA may reduce the risk of CRC in IBD^[63]. A post hoc analysis of a randomized, placebo-controlled trial of patients with UC and PSC suggested a significant chemoprotective effect for UDCA in these patients, with a 74% reduction seen in the risk for dysplasia or cancer in those assigned to the UDCA group^[64]. Tung *et al.*^[65] reported that UDCA use was strongly associated with a decreased prevalence of colonic dysplasia (OR: 0.18; 95%CI: 0.05-0.61). However, a retrospective analysis of data from a randomized controlled trial using a high dose UDCA (28-30 mg/kg body weight) showed that long-term use of high-dose UDCA was associated with an increased risk of colorectal neoplasia in patients with UC and PSC^[66]. The chemoprotective effect of using UDCA for patients with UC but without PSC has not been explored.

Low folate intake has been associated with an increased risk for developing CRC and colon adenoma in sporadic CRC^[27,56]. Folate deficiency is associated with alteration of the normal DNA methylation process, imbalance of steady-state levels of DNA precursors, and changes in chromosome and chromatin^[67]. Patients with IBD are at risk of folate deficiency because sulfapyridine, a moiety of sulfasalazine used in IBD therapy, can lead to an impairment of folate absorption^[68,69]. A meta-analysis showed that both sulfasalazine and folate supplementation have a protective effect in CRC development in patients with longstanding UC^[70]. However, recent studies failed to find a chemopreventive effect of folic acid supplementation^[56,60,71].

DIAGNOSIS AND SURVEILLANCE

One key element to decrease the risk of CRC in IBD may be the early diagnosis by colonoscopic surveillance and treatment of precancerous lesions^[10]. However, ran-

Table 2 Summary of the screening and surveillance recommendation from international guidelines for patients with inflammatory bowel disease

	ECCO 2008	BSG 2010	AGA 2010	ACG 2010
1 st screening	8-10 yr	10 yr	Max 8 yr	8-10 yr
Surveillance interval	Extensive: 2 yearly to 20 yr then annually Left sided: 2 yearly starting at 15 yr PSC: 1 yr	By risk: low 5 yr Intermediate 3 yr High 1 yr	1-3 yr More often at high risk <i>e.g.</i> , PSC	1-2 yr
Random biopsy	Recommended	Recommended	Recommended	Recommended
Chromoendoscopy	Superior to white light endoscopy	≥ 33 if no chromo	≥ 33	≥ 33

ECCO: European Crohn's and Colitis Organization; BSG: British Society of Gastroenterology; AGA: American Gastroenterological Association; ACG: American College of Gastroenterology; PSC: Primary sclerosing cholangitis. Modified from Ref [80].

domized controlled trials have not been performed to verify that surveillance colonoscopy is effective. A previous Japanese study reported that close surveillance results in the detection of 48% of the cancers, 61% of which are early cancers^[1,72]. A recent Cochrane analysis concluded that, for patients undergoing surveillance, cancers tend to be detected at an earlier stage and hence have a better prognosis, even if lead-time bias may contribute to the apparent benefit of surveillance^[1,73,74]. Indirect evidence appears to support an effectiveness of surveillance in reducing the risk of death from IBD-associated CRC and that surveillance may be acceptably cost-effective, although no clear evidence was shown that surveillance colonoscopy prolonged survival in patients with extensive colitis^[73,75].

Currently, several guidelines are available for recommending a specific surveillance program in IBD patients^[76-79]. Almost all the guidelines agree on the following: (1) that a screening colonoscopy should be performed on patients during clinical remission of the disease in order to avoid confusion of inflammatory changes with dysplasia; (2) that surveillance colonoscopy should be started 8-10 years after the onset of symptoms for patients with left sided or extensive colitis; (3) that regular surveillance schedules need to be followed after the initial colonoscopy; and (4) that two to four random biopsy specimens should be taken with a jumbo forceps every 10 cm along the entire colon, with additional samples being taken in suspicious areas. Some distinctions are also made, such as the surveillance interval. These are summarized in Table 2.

The success of surveillance colonoscopy will depend on improvement of the diagnostic yield of dysplasia or early colon cancer. As stated previously, many current guidelines recommend quadrantic random biopsies every 10 cm throughout the entire colon^[63,80]. However, a random biopsy only samples 0.03% of the mucosal surface and has a detection rate of < 2 per 1000 biopsies^[80,81]. A retrospective analysis estimated that 33 and 64 biopsy specimens are required to detect dysplasia with 90% and 95% probabilities, respectively^[78,82]. This has served as the basis for the surveillance practice recommendation, and current guidelines for dysplasia surveillance recommend a minimum of 33 biopsies^[10,78]. However, this biopsy protocol has failed to clearly demonstrate any significant

gain in mortality rates and cost effectiveness^[83-85]. Recent data have shown that most gastroenterologists do not follow the biopsy protocol. A previous study from the Netherlands showed that only 27% of gastroenterologists obeyed the recommended number of 33 random biopsies^[86]. Another study showed that more than 50% of the gastroenterologists surveyed obtained fewer than 10 colonic mucosal biopsies per endoscopic surveillance examination^[87,88].

The random biopsy protocol is now increasingly criticized, and increased focus is being placed on target biopsies supported by chromoendoscopy or other newer endoscopic techniques^[10,63,89]. Chromoendoscopy involves the topical application of a dye onto colonic mucosa to improve detection of subtle colonic lesions, characterization, or diagnosis that are not visible with white light endoscopy^[63,78,88]. Previous studies have demonstrated that the use of chromoendoscopy increases the detection rate of dysplasia by approximately 2 to 3 fold and gives a per lesion increase of 4 to 5 fold^[83,90,91]. Comparable diagnostic yields have been obtained with both methylene blue and indigo carmine^[90,92]. Although chromoendoscopy has a demerit of being more time consuming than white light endoscopy, recent guidelines have endorsed chromoendoscopy with target biopsies^[76,77,80,88]. Moreover, recent back-to-back colonoscopy studies have showed that withdrawal times were similar, at 10 min for colonoscopy with random biopsy and 11 min for chromoendoscopy with target biopsy. In addition, a targeted biopsy protocol with pancolonoscopic chromoendoscopy required fewer biopsies to detect dysplastic lesions^[90].

Narrow band imaging (NBI) is another available technique that can provide clear imaging of the microvascular structure. However, NBI was not any more efficacious than conventional colonoscopy in detecting patients with neoplasia in tandem study and multicenter randomized studies^[88,93-95]. Confocal laser endomicroscopy and fluorescence endoscopy are other promising techniques, but only limited data are available. Confocal laser endomicroscopy visualizes the histology of the mucosa and it has been proposed as an addition to chromoscopic detection of lesions to help target biopsy^[80,88]. A recent study showed that endomicroscopy targeted biopsies increased the diagnostic yield of intraepithelial neoplasias by 2.5-fold when compared to chromoendoscopy guided

biopsies alone^[96]. Kiesslich *et al.*^[83] demonstrated that the combined use of chromoscopy and endomicroscopy detected 4.75-fold more neoplasias than could be detected with conventional colonoscopy and 50% fewer biopsy specimens were required.

Fluorescence endoscopy assesses intraepithelial neoplasia after topical or systemic sensitization with 5-aminolevulinic acid (5-ALA), which is converted intracellularly into the sensitizer protoporphyrin IX and accumulates selectively in neoplastic tissue^[88,97]. In a previous study of 37 patients with UC, fluorescence endoscopy after 5-ALA sensitization showed excellent sensitivity, ranging from 87% to 100% after local sensitization, and negative predictive values of non-fluorescent mucosa for exclusion of dysplasia were also very high^[97]. A recent back-to-back study that used autofluorescence imaging, where short-wavelength light provided the excitation of endogenous tissue fluorophores, showed lower neoplasia miss rates for autofluorescence imaging than for white light endoscopy^[98].

CONCLUSION

Recent data about IBD-associated CRC have indicated several new trends. The overall incidence of CRC has been at a steady state or diminishing in western countries in recent decades. However, an anticipated increase in the occurrence of IBD-associated CRC has appeared in Asian countries as a consequence of the increasing incidence of IBD. Studies on the pathogenesis of IBD-associated CRC are now focused on genetic alteration. Interest is rising with regard to the role of several factors, such as oxidative stress, immune responses, and bacterial flora, in colon carcinogenesis in patients with IBD. Prospective randomized controlled trials on chemoprevention in patients with IBD are lacking and data on chemoprevention on patients with IBD are not clearly definitive; however, anti-inflammatory agents such as the 5-aminosalicylate compounds and immune modulators have been considered as potential chemopreventive agents. Several guidelines for surveillance strategy have also recently have endorsed the use of a target biopsy that incorporates chromoendoscopy or some new technologies instead of multiple random biopsies. However, the effectiveness of new technologies like narrow band imaging, confocal endomicroscopy, and autofluorescence imaging remain to be proven.

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Inflammatory bowel disease in pediatric and adolescent patients: A biomolecular and histopathological review

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childhood. It provides diagrams that display the main anatomo-clinical and biomolecular correlations and that may be encountered in IBD children. These diagnostic patterns and correlations may be useful in clinical practice for pediatric IBD.

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Abstract

Crohn's disease (CD) and ulcerative colitis (UC) are the two main forms of inflammatory bowel disease (IBD) with both overlapping and distinct clinical, pathological and biomolecular features. It has been suggested that pediatric IBD is a distinct disease entity, with probably different disease subtypes. The aim of this study is to review and summarize the evolution of the current concept of pediatric IBD. The results of this review reinforce the idea that pediatric CD and UC may be further classified in various clinicopathologic entities. For clinicians and pathologists convenience, practical algorithms for the distinction of the various subphenotypes of pediatric IBD are also provided.

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Key words: Pediatric inflammatory bowel disease; Crohn's disease; Ulcerative disease; Histopathology; Molecular biology

Core tip: The review contains the most recent data of the literature and suggests a clinical- pathological heterogeneity of the inflammatory bowel disease (IBD) in

INTRODUCTION

Inflammatory bowel disease (IBD) represents a group of idiopathic, chronic, inflammatory intestinal conditions in which complex interactions among genetic, immune, and environmental factors are involved. Crohn's disease (CD) and ulcerative colitis (UC) are the two most common forms of IBD with both overlapping and distinct clinical, pathological and biomolecular features. Traditionally, UC is defined as a disease involving the colonic mucosa in a diffuse, continuous manner, always affecting the rectum. In contrast, CD may involve any part of the gastrointestinal tract and frequently shows discontinuous or segmental involvement. However, IBD may be better considered as a syndrome of complex disorders with a significant heterogeneity in disease presentation and course^[1-5]. It has also been suggested that pediatric IBD is characterized by distinct phenotypic differences including disease type, disease location, disease behaviour, gender preponderance and genetically attributable risk, compared to adult-onset IBD^[6-11]. The incidence of IBD in childhood is rising worldwide^[12,13]. Rates are highest in North America and Europe, with rapid increases noted in developing nations adopting a Westernized lifestyle.

Childhood and adolescent IBD accounts for nearly 30% of total cases^[14-17]. Pediatric IBD demonstrates a pattern with CD predominating over UC. In particular, the incidence of CD has risen markedly, while a rather stable incidence of pediatric onset UC has been reported^[18]. A recent study based on the Swedish population found that the incidence of CD was 9.2 per 100000 per person years. The incidence of UC in children over the same time period was 2.8 per 100000 per person years^[19]. Similar rates have been reported by other studies^[20-24].

Recent population based studies have demonstrated a significant male excess in incidence of pediatric CD^[25]. Pediatric CD more often involves the ileocolonic/colonic regions, whereas adult CD does not demonstrate a high proportion of colonic disease^[7]. Furthermore, a variety of phenotypic characteristics have been described in pediatric UC thanks to increasing diagnostic accuracy^[26,27]. Reflecting these trends, IBD classification has been changed from the Vienna statement^[28], through the Montreal classification^[29] to the recent pediatric Paris classification^[30].

Although the precise etiology of IBD remains elusive, both animals and human studies point towards a strong genetic susceptibility. Genome wide association studies (GWAS) identified over 160 susceptibility loci/genes that are significantly associated with IBD^[31-33]. However, newer genomics technologies are now beginning to complement GWAS findings and add to our understanding of the molecular genetic universe of IBD. Genetic studies have provided detailed appreciation of the molecular architecture of IBD, and, in particular, the areas of overlap between CD and UC (such as Th17 pathways) and the pathways which are disease-specific. Moreover, the genes implicated in childhood-onset and adult-onset IBD often overlap, suggesting similar contributory genetic predisposition and pathophysiological pathways. For CD, gene discoveries have focused on defective processing of intracellular bacteria, autophagy, and innate immunity. For UC, the focus has been on barrier function.

A further addition to the complexity of understanding disease mechanisms is that a susceptibility allele often requires other genetic and non-genetic cues to manifest the disease^[34]. The variable concordance rate in monozygotic twins of 27%-50% in CD compared with 15%-19% in UC suggests that non-genetic factors may have an even more important role in UC than in CD^[35,36].

In the present review, we summarize current knowledge concerning the correlation between clinicopathologic features and genetic profiles of pediatric IBD in order to offer practical algorithms for the categorization of the vast majority in this group of lesions.

PARIS CLASSIFICATION

The issue of subclassification of IBD by phenotype has

been reviewed in recent years. The World Congress of Gastroenterology in Vienna, in 1998, considered age of onset (A), disease location (L), and disease behaviour (B) as predominant phenotypic elements^[28]. The Montreal revision of the Vienna classification did not change the three predominant parameters of age at diagnosis, location, and behaviour, but modification within each of these categories was made^[29]. However, the criteria of Montreal Classification has inherent limitations with respect to classification of pediatric IBD. In the pediatric Paris classification^[30] growth failure in the patient at any time was added as G1 *vs* G0 (never growth failure) to classic phenotypic elements (age at diagnosis, disease location, and disease behaviour). The comparisons between the Montreal and Paris classifications for CD and UC are shown in Tables 1 and 2, respectively.

IBD UNCLASSIFIED AND “INDETERMINATE” COLITIS

The term “indeterminate colitis” was originally coined for IBD resections with features of both UC and CD, usually in the setting of severe acute or “fulminant” colitis^[37]. Over the years the term has been adopted by clinicians to describe patients in whom a diagnosis of UC or CD cannot be made based on standard clinical testing, including colonoscopy, imaging, laboratory tests and biopsy^[38]. However, this term has been used incorrectly with considerable confusion among clinicians and pathologists. Recently, it has been recommended that the term “indeterminate colitis” be reserved only for patients for whom a surgical specimen is available and the term “colonic IBD type unclassified” (IBD-U) for patients with no surgical specimen available and for whom endoscopy is inconclusive and histological changes do not fit with either CD or UC^[29]. It remains controversial whether IBD-U constitutes a problem of classification or an IBD subtype distinct from CD and UC. Some authors believe that IBD-U is not a third form of IBD with specific diagnostic criteria, being a provisional diagnosis of exclusion used until a diagnosis of UC or CD is made with certainty^[39,40]. Instead, other authors consider IBD-U as a distinct phenotype of IBD for the following reasons: (1) A recent meta-analysis showed that IBD-U is more common in children accounting for 12.7% of all cases of IBD *vs* 6% in adults^[41]; (2) Children with IBD-U have a disease that rapidly progresses to pancolitis^[42,43]; (3) Although many patients with IBD-U will be reclassified as having CD or UC on long-term follow-up evaluation, a significant proportion of them will still carry the diagnosis of indeterminate colitis^[44]; (4) Epidemiologic data have shown that clinical course and prognosis of IBD-U could be worse compared with UC, especially concerning outcome of surgery with greater risk of pouchitis^[45]; and (5) As will be discussed in the next section, IBD-U is diagnosed in a large subgroup of patients at a very

Table 1 Montreal and Paris classifications for Crohn's disease

Montreal classification				Paris classification	
Age at diagnosis	A1	< 17 yr	A1a	< 10 yr	
			A1b	10-16 yr	
	A2	17-40 yr	A2	17-40 yr	
	A3	> 40 yr	A3	> 40 yr	
Location	L1	Ileal disease	L1	Distal 1/3 ileum ± limited cecal disease colonic disease ileocolonic disease isolated upper disease	
	L2	Colonic disease	L2		
	L3	Ileocolonic disease	L3		
	L4	Isolated upper disease	L4		
			L4a	Upper disease proximal to ligament of Treitz	
			L4b	Upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum	
Behavior	B1	Non-stricturing, non-penetrating	B1	Non-stricturing, non-penetrating	
	B2	Stricturing	B2	Stricturing	
	B3	Penetrating	B3	Penetrating	
			B2B3	both stricturing and penetrating	
	P	Perianal disease modifier	P	Perianal disease modifier	
Growth	Not applicable		G0	No evidence of growth delay	
			G1	Growth delay	

Table 2 Montreal and Paris classifications for ulcerative colitis

Montreal classification				Paris classification	
Extent	E1	Ulcerative proctitis	E1	Ulcerative proctitis	
	E2	Leftsided colitis distal to splenic flexure	E2	Leftsided colitis distal to splenic flexure	
	E3	Extensive colitis distal to hepatic flexure	E3	Extensive colitis proximal to splenic flexure	
Severity	S0	Clinical remission	E4	Pancolitis, proximal to hepatic flexure	
	S1	Mild ulcerative colitis	S0	Never severe	
	S2	Moderate ulcerative colitis	S1	Ever severe	
	S3	Severe ulcerative colitis			

early age (0-2 years, infantile IBD)^[30-46].

AGE OF ONSET

An important modification recommended by the Paris classification^[30] includes age at onset as A1a (0 to < 10 years) and A1b (10 to < 17 years). In both the Montreal^[29] and Paris^[30] Classification systems, A2 and A3 account for age of diagnosis at 17-40 years, and > 40 years, respectively.

Although rare, IBD may occur before the age of 2 years. Therefore, the Paris classification^[30] suggested the possibility of distinguishing a separate group of children diagnosed with IBD at a very early age (0-2 years, infantile IBD). This subgroup is characterized by a high rate of consanguinity, more severe disease course, association with primary immunodeficiency and resistance to immunosuppressive treatment^[45,46]. Abscess formation, anal fissures and enterocutaneous or rectovaginal fistulae complicate the disease and frequently require partial or total colectomy^[30,47].

The suspicion of a monogenetic cause of these early onset forms was recently confirmed by the discovery of mutations in the genes coding for one of the two IL10 receptors causing impaired IL10 signalling^[48-52].

The region encoding IL10 was originally identified by a German GWAS in UC, and the association docu-

mented with non-coding variants upstream of the IL10 gene^[53]. Subsequently, an international GWAS meta-analysis showed that this region is associated with CD^[54]. Through a genetic-linkage analysis and candidate-gene sequencing on samples from two unrelated consanguineous families with children affected by early-onset IBD, Glocker *et al.*^[55] identified three distinct homozygous mutations in genes IL10RA and ILRB. These genes encode the IL10R1 and IL10R2 proteins, respectively, which form a heterotetramer to make up the IL10 receptor. Functional experiments have shown that the *IL10RA* and *ILRB* gene mutations abrogate IL10 signalling and lead to severe intestinal inflammation. Loss of function mutation in IL10 and IL10R was also identified in 66 patients with very early onset IBD. In this study, it has been found that in 5 patients with IL10R deficiency, the allogenic hematopoietic stem cell transplantation induced sustained clinical remission with a median follow-up time of 2 years^[49]. Recently, Moran *et al.*^[50] have identified a novel homozygous, splice-site point mutation in IL10RA in an infantile-onset IBD Caucasian female. The patient was also affected by significant arthritis and folliculitis. The mutation caused a premature stop codon (P206X) and IL10 insensitivity. Moreover, 188 children with early-onset IBD and 188 healthy subjects have been studied. In the discovery cohort, five IL10RA polymorphisms associated with UC have been found^[50]. These studies

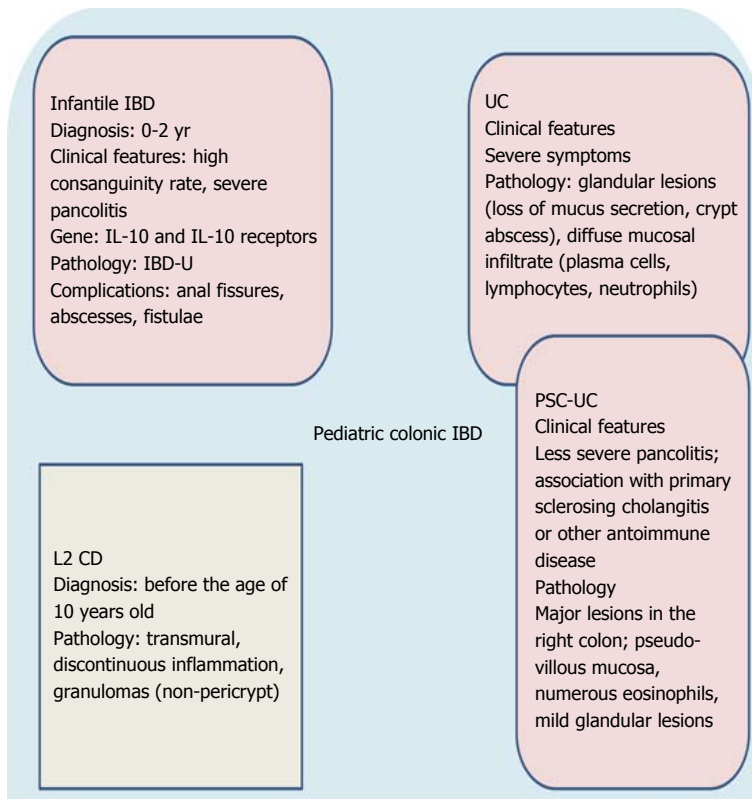


Figure 1 Distinct clinicopathological and biomolecular features of the pediatric colonic inflammatory bowel disease. IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

show the role of immune pathways in early onset IBD pathogenesis. Indeed, IL10 is an anti-inflammatory cytokine secreted by a variety of cell types and is critical for maintaining immune homeostasis in the gastrointestinal (GI) tract. IL10 restricts excessive immune response^[56]. In particular, IL10 limits the secretion of proinflammatory cytokines, such as tumour necrosis factor α (TNF- α) and IL12^[57]. Moreover, the assembly of IL10R1 results in the activation of the receptor-associated Janus tyrosine kinases, JAK1 and Tyk2, leading to the phosphorylation of STAT3 (signal transducer and activator of transcription 3) and the induction of STAT3-dependent genes^[58]. A severe enterocolitis has been found in mice that are deficient in either IL10 or IL10R2. These data underline the pivotal role of IL10 in the mediation of signalling that controls inflammation in the gut.

Other monogenic primary immunodeficiencies showing IBD-like gastrointestinal pathology include Wiskott-Aldrich syndrome^[47], chronic granulomatous disease^[47], XIAP deficiency^[55,59], X-linked (IPEX) syndrome^[60] and nuclear factor κ B essential modulator (NEMO) deficiency^[61]. It is also noteworthy that a large subgroup of these patients have IBD-U^[30,42]. Therefore, we think that IBD-U is an IBD subtype distinct from CD and UC as it constitutes a histopathological substrate of a clinicopathologic entity with characteristic epidemiological (< 2 years old), clinical (severe clinical course with pancolitis), and genetic features (*i.e.*, mutation in interleukin-10 receptor and interleukin gene or other primary immuno-

deficiencies). It is, therefore, plausible that infantile IBD (< 2 years) may be incorporated into future modifications of the Paris classification. Figure 1 summarizes the main clinicopathologic characteristics of infantile IBD.

CD

Disease location

According to the Paris classification^[30], CD location is categorized as follows: (L1) involvement of the terminal ileum only, with limited or no cecal disease; (L2) colonic involvement only; (L3) involvement of both the terminal ileum and colon; (L4) isolated upper gastrointestinal disease. L4 is further separated into esophagogastrroduodenal disease (L4A), jejuna/proximal ileal disease (L4B), or both L4A and L4B. Adolescents more often present with ileal disease (L1), whereas children have a tendency to present with isolated colonic disease (L2). Pediatric patients with L2 disease are less likely to have esophagogastrroduodenal involvement or stricturing disease behaviour than patients with L1 and L3 disease^[62]. These data support the existence of discrete subtypes of CD, which are, in part, defined by the predominant anatomical location of disease^[7] and summarized in Table 3 and Figure 2.

Paneth cells are protagonist in the L1CD

Histologic, immunologic and biomolecular evidence strongly suggests a key role for Paneth cells in L1 CD. Histologically, Paneth cells are abundant in the small

Table 3 Salient histopathological and biomolecular features in pediatric inflammatory bowel disease

Gene	Genetic alterations	Histopathology	Clinical features	Ref.
<i>IL10RA</i>	G141R	IBD-U	Infantile IBD	[48-50]
<i>IL10RB</i>	W159X			
<i>NOD2</i>	R702W, G908R, L1007fsinsC	Abnormal Paneth cells, granuloma-poor	L1 CD	[72]
<i>ATG16L1</i>	T300A			
<i>NOD2</i>	R702W, G908R, L1007fsinsC		L1 CD, Structuring (B2) behavior	[81-87] [82,83,87]
			Early surgery	[83]
			Growth delay	[83,84]
			Higher disease activity	[83,88]
<i>NOD2</i>	R702W, G908R, L1007fsinsC	Diminished immunohistochemical Expression of alpha-defensin in small intestinal Paneth cells	L1, L3 CD	[106-108]
<i>TLRs</i>	TLR-4 Asp299gly		CD, UC	[111]
<i>IRGM</i>	rs4958847		Fistulizing (B3) CD	[135]
<i>ILRL1</i> and <i>IL33</i>	Upexpression of mRNA		Extensive UC	[138-140,150]
β -defensin	Low gene copy number	Diminished immunohistochemical expression of beta-defensin 2	L2 CD	[104,105]
<i>HBD2</i>				
<i>NCF4</i>	Rs4821544 polymorphism		Perianal CD	[157]

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis.

intestine and are occasionally found in the cecum and proximal ascending colon^[63]. In parallel, L1 (distal 1/3 ileum \pm limited cecal disease) CD location corresponds to the normal distribution of Paneth cells.

From an immunological viewpoint, Paneth cells, by virtue of their vast repertoire of effector molecules, are multifunctional cells. They produce antimicrobial proteins such as alpha-defensins HD5 and HD6, lysozyme, secretory phospholipase A2 and the lectin Reg III γ ^[64]. Wehkamp *et al.*^[65] showed that ileal (L1) but not isolated colonic (L2) CD is associated with a diminished synthesis of Paneth cell defensins. Wehkamp and Stange^[66], therefore, proposed the term "Paneth's disease" to describe a complex disease in which Paneth cells might explain the poor antimicrobial capability of (L1) CD. Paneth cells have also been shown to contain TNF- α transcripts. With ultrastructural immunogold methods, Beil *et al.*^[67] showed TNF- α in mature and immature secretory granules of Paneth cells. One of the major biological roles of TNF- α is in the host defence to bacterial, viral and parasitic infections, and Paneth cells are considered to be the major source of TNF- α in the normal bowel^[67]. Clinical and molecular studies implicate TNF- α as a key mediator in the initiation and propagation of CD^[68]. This is evidenced by an increased amount of TNF in inflammatory cells infiltrating ileal tissue (eosinophils, mast cells, neutrophils, macrophages, fibroblasts) and a marked clinical response of TNF- α antagonists in patients with active CD^[69]. Therefore, abnormal production of TNF- α protein by Paneth cell may be involved in the pathogenesis of L1 CD^[69].

From a histopathological viewpoint, Paneth cell depletion occurs in the most heavily inflamed areas of the ileal mucosa^[70]. Recently, Günther *et al.*^[71] showed a significant decrease in the number of Paneth cells and a high number of dying cells with shrunken eosinophilic cytoplasm at the crypt base in histological samples from terminal ileum of patients with active CD. Paneth cells showed ultrastruc-

tural signs of non-apoptotic cell death, such as organelle swelling, vacuole formation and the lack of blebbing. The data of Günther *et al.*^[71] suggest that necroptosis (a form of non-apoptotic cell death) of Paneth cells might be involved in the pathogenesis of CD.

Recently, VanDussen *et al.*^[72] have studied the correlation between Paneth cell phenotype (based on lysozyme-positive secretory granule morphology) and NOD2 and ATG16L1 genotype. They observed an inverse correlation between abnormal Paneth cells (with disordered, diminished, diffuse, or excluded granule phenotypes) and the presence of granulomas^[72]. The cumulative number of NOD2 and ATG16L1 risk alleles had an additive effect on the proportion of abnormal Paneth cells. Moreover, high proportions of abnormal Paneth cells were associated with shorter time to disease recurrence after surgery. VanDussen *et al.*^[72] suggest stratifying CD based on Paneth cell phenotypes rather than the presence of granulomas. In fact, granulomas could be sparse and more likely to be undersampled, especially in biopsy specimens, whereas Paneth cell phenotypes are more easily analyzed within limited samples. Therefore, these authors concluded that histologic analysis of Paneth cell phenotypes can be used to divide patients with CD into subgroups with distinct pathognomonic and clinical features^[49]. However, further correlative histopathologic and biomolecular studies are necessary to clarify the role of necroptosis and/or granule changes of Paneth cells in L1 CD characterized by abundant or few granulomas (so-called granuloma-rich or granuloma-poor CD).

Biomolecular studies of antimicrobial function in Paneth cells

Defective antimicrobial function in Paneth cells has been described by a variety of mechanisms including mutations in the innate immune receptor NOD 2, defensins, Toll-like receptors (TLRs), the autophagy protein ATG16L1, *IRGM* gene.

NOD2 gene

With the pivotal study of Hugot *et al.*^[73], who discovered the very first and also strongest susceptibility gene for CD in NOD2/CARD15, “IBD gene hunting” was opened. NOD2 is an intracytoplasmic member of the family of intracellular NLR (NOD like receptors) able to recognize pathogen associated molecular patterns (PAMPs) and to modulate an inflammatory response through enhanced NF- κ B activation^[74,75]. NOD2 is highly expressed in cells of the phagocyte system and is involved in the production of defensins in response to gut microbiota. Three single nucleotide polymorphisms (SNPs) within the NOD2/CARD15 gene (R702W, G908R, and 1007fsinsC) have been established as independent risk factors for CD in Caucasians^[76,77] and they represent 82% of the mutations in NOD2^[78]. Interestingly, the NOD2/CARD15 mutations are absent or very rare in Asians, Arabs, Africans, and African Americans^[79]. NOD2/CARD15 variants have been associated to more severe CD, a greater need for surgery and a younger age at onset^[80]. In children, a correlation with ileal (L1) localization^[81-87], stricturing behavior^[82,83,87], early surgery^[83], growth delay^[83,84] and higher disease activity^[83,88] has been found. However, these results have not been confirmed by other studies^[89-91]. The precise mechanistic relationship between NOD2/CARD15 and CD remains controversial. Interestingly, a study by Zelinkova *et al.*^[92] suggested that NOD2 mutations may result in perpetuation of mucosal inflammation through insufficient pathogen elimination.

The important role of NOD2 in the pathogenesis of CD has been underlined by recent studies on microRNAs (miRNAs)^[93]. miRNAs are short non-coding RNAs that have emerged as key modulators of various cellular processes at the post-transcriptional level^[94-96]. There are recent reports on their role in the regulation of intestinal permeability as the loss of intestinal miRNAs impairs the epithelial barrier function, and causes acute inflammation^[97]. Some Authors showed that miRNA-122 regulates intestinal permeability tight junctions (TJ) by targeting occluding mRNA degradation^[98-102]. Moreover, Chen *et al.*^[102] found that NOD2 is a functional target of miRNA-122.

Other mechanisms have been proposed for NOD2/CARD15 mutations in recent years, ranging from abnormal Paneth cell function with reduced defensin secretion, altered modulation of Toll-like receptor signaling and a reduced ability to trigger autophagy^[103]. Several studies have shown the role of defensins in the pathogenesis of CD^[104-107]. In particular, ileal (L1) and ileal colonic (L3) CD is characterized by a specific decrease in small intestinal Paneth cell human α -defensin HD-5 and -6^[106-108]. In a group of pediatric CD, Perminow *et al.*^[109] studied the role of HD5 and TCF-4, a Wnt-signaling transcription factor which controls Paneth cell defensin expression. They showed a low intestinal expression of HD5 and TCF4 mRNA in ileal CD, confirming the important role of antimicrobial host defense in pediatric patients.

Toll-like receptors (TLRs) have an important role in the pathogenesis of CD. They are crucial components of innate immunity and cell surface molecules that also detect normal and pathogenic microbial agents and can trigger antimicrobial host defense responses. TLRs are abundantly expressed on the surface of monocytes, macrophages, and dendritic and epithelial cells^[110]. In IBD mucosa, dendritic cells are activated and there are increased levels of TLR2 and TLR4. These TLRs mediate the recognition of bacterial lipoproteins and LPS, respectively. An association has been described between the TLR4 D299G SNP and IBD, in UC as well as CD patients^[111]. This SNP is associated with impaired LPS signaling and increased susceptibility to gram-negative infections, thus supporting the role of gram-negative bacteria in the pathogenesis of IBD. Polymorphisms have been described in IBD with regard to the TLR9 gene, but their functional significance is still unclear^[112].

Genes of the autophagy: A key role in IBD

The autophagy 16-like 1 (*ATG16L1*), immunity-related guanosine triphosphate M (IRGM), and leucine-rich repeat kinase 2 (*LRRK2*) genes, which regulate autophagy, have been associated with CD in GWAS^[113,114].

Autophagy describes the sequestration of intracellular material such as obsolete organelles or large unfolded protein within membranes, and their trafficking to fuse with lysosome with a subsequent degradation of the contents. Autophagy is now recognized as playing a key role in innate immunity against intracellular microorganisms.

ATG16L1 is essential for all forms of autophagy as it has a role in the clearance of intracellular bacteria^[115]. It interacts with two other autophagy proteins, ATG5 and ATG12, to form a complex essential for the process of autophagy^[116]. The association at ATG16L1 with CD seems to be entirely accounted for by the *T300A* coding variant which maps to a highly conserved part of the gene adjacent to the coiled-coil domain. ATG16L1 mutations cause a deficiency of the correspondent protein and disrupt the recruitment of the ATG12-ATG5. Therefore, autophagosome formation and degradation of proteins with a long half-life are severely impaired in ATG16L1-deficient cells^[117,118]. The decreased autophagy impairs immune tolerance by autoantigen presentation on major histocompatibility complex class II molecules and causes immune inflammation^[119].

ATG16L1 gene mutations could also impair the mechanisms that involve autophagy and apoptosis as there is an acceleration in the rate of epithelial cell apoptosis and an inhibition of inflammatory cell apoptosis in CD and UC^[120,121]. Several studies have been performed on the role of *ATG16L1* gene in the pathogenesis of IBD.

The *T300A* allele was correlated with the incidence of CD in three populations from Germany, Hungary and the Netherlands^[122]. In contrast, no correlation between *T300A* allele and UC was detected. However, the results of other studies on the association of *T300A*

allele with predisposition to CD and UC, are inconsistent^[122], also in child-onset IBD cases^[123]. Recently, a meta-analysis performed on twenty-five studies of CD, 14 of which involved cases of UC^[124] showed that the T300A allele confers a susceptibility to CD and to UC. However, ATG6L1 was associated with the risk of child-onset CD, but not with child-onset UC probably because there are few studies on pediatric UC. Recently, a functional link between NOD2/CARD15, ATG16L1 and autophagy has been provided^[125-128].

IRGM gene

In the Wellcome Trust Case-Control Consortium (WTCCC) GWA scan, a highly significant association between variant flanking IRGM and susceptibility to CD has been shown. The IRGM gene is located on chromosome 5q33.1, and is required during the initiation phase of autophagy, when it localizes to bacteria-containing autophagic vacuoles. IRGM and autophagy are involved in clearance of intracellular organisms such as *M. tuberculosis*^[129,130] and the CD-associated IRGM variant is predicted to affect autophagic control of *Salmonella typhimurium*^[131].

The IRGM risk alleles for CD are non-coding and appear to affect mRNA transcription or stability. Over the last 5 years three distinct mechanisms have been identified which might explain the impact on IRGM expression. Cooney *et al.*^[131] discovered a large copy number variant upstream of IRGM which correlated with tissue-specific expression effects. Prescott *et al.*^[132] reported a disruption of a transcription factor binding site in the IRGM promoter. Most recently, Brest *et al.*^[133] found that a synonymous coding variant of IRGM alters the binding domain of miRNA196, a family of miRNAs, hence affecting mRNA stability and gene translation. In this study, it has been shown that microRNA196 is overexpressed in CD patients and that it downregulates an IRGM protective variant but not the risk-associated allele.

Lapaquette *et al.*^[134] found that the reduced IRGM gene expression correlated with impaired clearance by macrophages of CD-associated adherent-invasive *Escherichia coli*. A recent Italian study^[135] reported the association between CD and two risk SNPs (rs1000113 and rs4958847) for IRGM, irrespective of age, but not in UC. In addition, a trend to B3 (penetrating) disease behavior in patients with IRGM SNPs was suggested^[135]. Moon *et al.*^[136] showed that IRGM SNP rs10065172 was significantly associated with CD susceptibility. They also reported a protective role of SNP rs72553867.

By suppressive subtractive hybridization (SSH) technique, Sim *et al.*^[137] studied the differential expression gene profiles in ileal biopsies from CD children. Twenty-eight genes previously reported in association with adult CD, and 47 new genes were identified. It is significant that some adult CD genes have also been found in early-onset CD. Indeed, it underlines that, in some cases, there is a common genetic pathway between pediatric and

adult CD cases. Several genes reported in the study are involved in microbial pathogenesis, antigen presentation, inflammation, regulation of epithelial barrier function, vesicular transport or cell differentiation and proliferation. Recent studies have found that IL33 expression is enhanced in the inflamed colonic mucosa of IBD, especially in UC^[138,139], and the IL33/IL1RL1 signalling axis has been implicated in the IBD pathogenesis^[140]. Recently, new genes have been identified by GWAS in IBD^[141-150]. In particular, in 2008, Kugathasan *et al.*^[141] performed the first GWAS in a cohort with pediatric disease onset, identifying two new loci on 20q13 and 21q22. This paper reported the associations with two intronic SNPs (rs2315008 and rs4809330) at the 20q13 locus. These two SNPs map to the zinc finger CCCH-type with G patch domain (ZGPAT) gene which is located in a region containing eight potential candidate genes for CD. Moreover, an association, at the 21q22 region, of CD with the intergenic SNP rs2836878 has been shown that implicated the nearby PSMG1 gene^[141].

A follow-up scan on an extended cohort of 3426 childhood-onset IBD (European/North America collaboration) identified five more new loci associated with pediatric IBD^[147]. These loci included 16p11 near the cytokine gene IL27, 22q12, 10q22, 2q37 and 19q13.11. The results of this study showed that IL27 is a promising candidate gene for pediatric CD. Recently, Latiano *et al.*^[149] have studied a large Italian cohort of adult and early-onset IBD to verify the role of new genes involved in the immune response and inflammation (PTGER4, HLA-BTNL2, TNFSF15, NKX2-3, ZNF365, IFNG, PTPN2, and PSMG1).

Role of colonic epithelial cells producing β -defensins in colonic (L2) CD location.

Since Paneth cells are rare in the colon, the contribution of α -defensins to antimicrobial defence in the large intestine is only limited. In contrast, β -defensins HDB1-3 are secreted by columnar and goblet cells in the colon^[151,152]. A deficiency of β -defensin HDBD2 has been shown by molecular biology in L2 (colonic) CD and confirmed on the protein level by immunohistochemistry^[104]. This observed colonic defect of the antimicrobial barrier caused by a diminished expression of β -defensins may allow luminal microbes to attach to and invade the mucosa triggering the inflammation^[153].

CD behaviour

The Montreal classification^[29] describes three behaviours for CD: nonstricturing nonpenetrating disease (B1), stricturing disease (B2), penetrating/fistulizing disease (B3). In addition, the Paris classification^[30] proposes a new classification B2B3 to identify patients with both B2 and B3 phenotypes (either at the same or different times). Disease locations are the most important factor identified in determining the risk of developing either a structuring or penetrating complication (more complications with ileal, less with colonic disease)^[154]. To date,

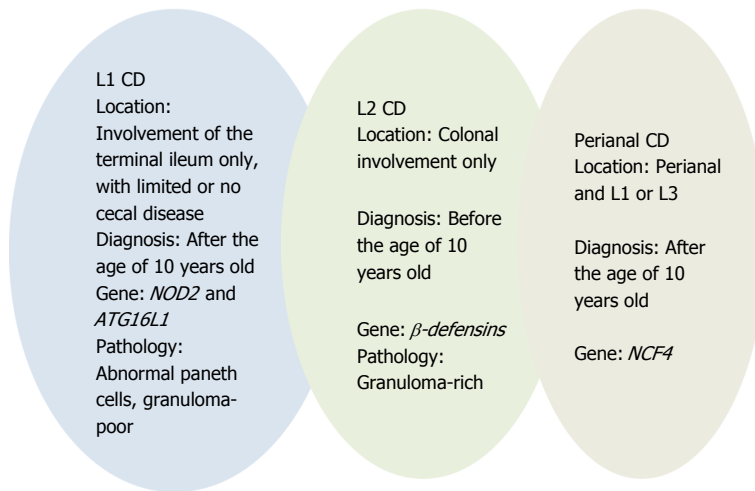


Figure 2 Subtypes of Crohn's disease. CD: Crohn's disease.

there have been few pediatric studies that have evaluated the association between CD behavior and genotype with prolonged follow-up. In a study by Shaoul *et al.*^[155], pediatric CD at the end of follow-up (mean 4.9 years) was classified as inflammatory (78%), stricturing (17%) and penetrating (7%). Moreover, a role for *NOD2* in CD behavior (as opposed to location) was not supported in this study. *NOD2* genotype was clearly associated with ileal involvement (L1), but not as an independent risk factor for stricturing, penetrating disease or a need for surgery^[155]. A recent study showed that L1 CD and stricturing disease behavior are more common in children diagnosed after 10 years of age than in younger patients^[39].

Approximately 10% of newly diagnosed pediatric patients shows perianal CD at time of diagnosis^[156]. The Vienna classification did not distinguish luminal and perianal fistulising disease into different categories^[28]. Subsequent evidence suggested that perianal and luminal fistulae often occur completely independently of each other. According to the Montreal and Paris classifications^[29,30], a separate perianal modifier was added that can coexist with any disease behaviour. Perianal CD is defined as inflammation at or near the anus, including tags, fissures, fistulae, abscesses, or stenosis. A recent study by Eglinton *et al.*^[157] suggests specific associations with perianal CD patients compared with patients without perianal involvement. These associations include younger age at diagnosis, male gender, ileal (L1 or L3) location, and complicated disease behaviour (B2 + B3). In addition, an association with the *NCF4* gene was demonstrated^[157]. Taken together, literature data suggest that perianal CD represents a distinct disease phenotype, summarized in Figure 2.

UC

UC is classically defined as a chronic inflammation characterized by a continuous involvement of the colonic mucosa without granulomas on biopsy, affecting the rectum and a variable extent of the colon in continuity,

and characterized by a relapsing, remittent course^[158]. However, several studies suggest that pediatric UC may present with atypical phenotypes, such as macroscopic rectal sparing, macroscopic skip lesions in the colon, periappendiceal inflammation, backwash ileitis, limited upper gastrointestinal inflammation, and extensive colitis with less severe and less diffuse architectural abnormalities^[159,160]. Thus, diagnosis of UC in pediatric patients may be particularly difficult. The recent Paris classification^[30], which examined this issue, adds a new category (E4) for pancolitis (inflammation extending proximal to the hepatic flexure) (Table 2). Moreover, a disease behavior classification of S0 or S1 was adopted, with the latter denoting the presence of severe disease at any time in patient history (Table 2). The classification of UC based on anatomical extent has clinical relevance, as it affects the choice of therapy and the mode of delivery of drugs. For example, oral therapy is the first choice for UC extending above the splenic flexure^[161].

Primary sclerosing cholangitis (PSC) is a cholestatic liver disease of unknown etiology, commonly associated with IBD and characterized by inflammation, fibrosis, and stenoses of the biliary tree leading to liver cirrhosis^[162]. In most patients (80%-90%) the IBD can be classified as UC. Several features of UC in PSC differ from those of a general UC population (increased frequency of pancolitis, "backwash ileitis", rectal sparing and colorectal carcinoma), and these observations have led to the hypothesis that UC in PSC may represent a specific phenotypic entity (PSC-UC)^[163]. There are a few studies on PSC-UC in pediatric patients. Ordonez *et al.*^[164] studied twenty-eight consecutive children with UC associated with PSC, celiac disease, or autoimmune hepatitis comparing them with a matched control group of 27 children with isolated UC. At diagnosis, pancolitis was seen in 18/28 UC associated with autoimmunity patients compared with 8/27 in UC. Pathological findings were also different from isolated UC: (1) major lesions predominantly located in the right colon; (2) pseudo-villous appearance of the mucosa, and strong infiltration

with eosinophils; and (3) mild glandular lesions. Evolution in UC associated with autoimmune disease was less aggressive, requiring less corticosteroids/immunomodulators^[164]. In conclusion, clinical, histological, and molecular analyses reveal marked differences between pediatric patients with isolated UC and those with associated autoimmune phenomena, supporting the hypothesis of a distinct autoimmune presentation of UC (Figure 1).

Goblet cells as protagonist in the UC

Mucus produced by goblet cells forms a key component of the mucosal barrier. Gel-forming mucins of intestinal mucus are arranged into a bilayer with a firm inner layer devoid of bacteria and a looser outer layer with MUC2 being major constituent of both^[165]. The mucus layer has a crucial role in intestinal homeostasis, as decreased levels of goblet cells, leading to reduced mucin secretion, are a hallmark of human UC^[166]. The thickness of the mucus layer in the healthy colon is between 100 and 300 μm , increasing from the ascending colon to the rectum, whereas in UC, this mucus layer is thinner, more variable and in part denuded^[167-169]. Taken together, several lines of evidence strongly suggest a key role for goblet cells in UC.

Molecular biology of UC

GWAS and candidate gene association studies identified several UC susceptibility loci, including 7 that overlap with CD (*e.g.*, IL23 pathway genes, NKX2-3 and IL10)^[170]. In a recent study, three new UC specific loci (*HNF4A*, *CDH1* and *LAMB1*) has been found that are involved in the regulation of barrier function^[171].

An association was seen at rs6017342 SNP which maps within a recombination hotspot on chromosome 20q13 in which the 3' untranslated region (UTR) of the *HNF4A* gene is located. This gene encodes the transcription factor hepatocyte nuclear factor 4 α which regulates the expression of multiple components within the adherens junction, the tight junction and desmosome^[172].

Cell-cell junctions have an important role on epithelial organization and barrier function. Moreover, in the embryonic age, the *HNF4A* gene participates in the development of mammalian gastrointestinal tract^[173]. *CDH1* gene which encodes E-cadherin has been located on chromosome 16q22. E-cadherin is a transmembrane glycoprotein, a key component of the adherens junction and mediates intercellular adhesion in the intestinal epithelium^[171]. It also participates in processes of epithelial restitution and repair following the damage of mucosa. Indeed, there is a significant reduced expression of *CDH1* in areas of active UC^[174]. Interestingly, two *CDH1* variants associated with UC^[171] have also been associated with colorectal cancer^[175], a possible complication of UC. The recent hypothesis is that *HNF4A* and E-cadherin co-participate to maintain the integrity of the epithelial intestinal barrier. *LAMB1* gene is located on chromosome 7q31^[171] and encodes the laminin β 1 subunit, which is detected in laminins-1, -2 and -10. In

UC, the expression of laminins in the intestinal basement membrane is downregulated^[171].

In 2011, Anderson *et al.*^[176] identified 29 additional UC risk loci from a meta-analysis and *IL1R2*, *IL8RA/B*, *IL7R*, *IL12B*, *DAP*, *PRDM1*, *JAK2*, *IRF5*, *GNA12* and *LSP1* were proposed as new important candidate genes in UC pathogenesis. Several genes were associated with cytokines and cytokine receptors, key regulators of cytokine-mediated signalling pathways, innate and adaptive immune response, macrophage activation and regulation of apoptosis. Interestingly, an association with *DAP* gene (death-associated protein) has been found. The expression of *DAP* kinase increases with inflammation in UC^[177] and is a negative regulator of autophagy^[178]. Therefore, the association with *DAP* suggests a possible link between autophagy and UC.

PRDM1, *IRF5* and *NKX2-3* genes could have a key role for transcriptional regulation in UC pathogenesis. *GNA12* which plays a fundamental role in the tight junction assembly in epithelial cells has been identified at the 7p22 locus^[179]. It is the most likely UC candidate gene from those described in the meta-analysis of Anderson *et al.*^[176]. Barrier integrity is important in UC pathogenesis given previous associations to *HNF4A*, *CDH1* and *LAMB1* genes^[171]. Further studies are, however, needed to verify the role of the genes in pediatric UC. The salient histopathological and biomolecular features of pediatric IBD are shown in Table 3.

CONCLUSION

In conclusion, our review reinforces the idea that pediatric UC and CD may be further classified into various clinicopathologic entities, based on genotype-phenotype correlation reported in recent literature. Therapy for complex genetic diseases such as IBD is difficult. For example, treatment with anti-TNF- α monoclonal antibodies does not induce remission in the majority of CD cases. It is becoming increasingly apparent that novel strategies to define and stratify IBD patients, that are based on serum, DNA and histopathology, will be needed to progress towards improved diagnostics, prognostics and therapeutics.

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Non-pulmonary allergic diseases and inflammatory bowel disease: A qualitative review

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Abstract

While the etiological underpinnings of inflammatory bowel disease (IBD) are highly complex, it has been noted that both clinical and pathophysiological similarities exist between IBD and both asthma and non-pulmonary allergic phenomena. In this review, several key points on common biomarkers, pathophysiology, clinical manifestations and nutritional and probiotic interventions for both IBD and non-pulmonary allergic diseases are discussed.

Histamine and mast cell activity show common behaviors in both IBD and in certain allergic disorders. IgE also represents a key immunoglobulin involved in both IBD and in certain allergic pathologies, though these links require further study. Probiotics remain a critically important intervention for both IBD subtypes as well as multiple allergic phenomena. Linked clinical phenomena, especially sinonasal disease and IBD, are discussed. In addition, nutritional interventions remain an underutilized and promising therapy for modification of both allergic disorders and IBD. Recommending new mothers breastfeed their infants, and increasing the duration of breastfeeding may also help prevent both IBD and allergic diseases, but requires more investigation. While much remains to be discovered, it is clear that non-pulmonary allergic phenomena are connected to IBD in a myriad number of ways and that the discovery of common immunological pathways may usher in an era of vastly improved treatments for patients.

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Key words: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Food intolerance; Food allergies; Biomarkers; Pathophysiology; Nutrition; Probiotics

Core tip: There are multiple clinical, pathophysiological and therapeutic commonalities between nonpulmonary allergic disease and inflammatory bowel disease (IBD). In particular, in terms of pathophysiology, histamine expression is upregulated in both IBD and allergic diseases. Ulcerative colitis, in particular, shows upregulation of the Th2 pathway which is seen in a large number of allergic phenomena including sinonasal disease. Both probiotics and nutritional interventions are promising therapies for both IBD and allergic disease (especially food intolerance, food allergies, and eczema) but these require more investigation. Recommending mothers breastfeed their infants, and for a longer duration also shows potential promise in prevention of both IBD and food allergies, but also requires further study.

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INTRODUCTION

Inflammatory bowel disease (IBD) is comprised of two major disorders, ulcerative colitis and Crohn's disease (CD). The exact pathophysiology of IBD remains unclear; however immune dysregulation plays a substantial role, with likely significant involvement of the Th1 and Th17 pathways in Crohn's and the Th2 pathway in ulcerative colitis. Intriguingly, some clinical manifestations of allergic disorders and IBD overlap, as well as expression of selected cytokines and immune responses. In particular, both disorders feature histamine release and IgE overexpression. Certain probiotics have been found to be useful in both disorders. There have been some studies that have shown a correlation between sinonasal allergic disease and IBD. Moreover, some food allergies and intolerances have been linked to IBD. Finally, sulfasalazines are often used to treat patients with IBD; such therapy can require desensitization for an individual patient to successfully use. It is evident that allergic disorders and IBD share common etiological characteristics and also share potential common treatment pathways.

HISTAMINE AND OTHER BIOMARKERS ASSOCIATED WITH IBD AND ALLERGY

Role of histamine and mast cells

In 1978, Dvorak *et al*^[1], followed by Levo and Livni^[2], found ultrasonographic and morphological evidence of degranulation of mast-cells in the submucosa of ileal specimens from patients with CD. They found release of mediators including histamine, platelet activation factor, and eosinophil chemotactic factor, all of which may play a role in the pathophysiology of CD. Subsequently, Dvorak *et al*^[1], using transmission electron microscopic studies discovered a markedly increased number of mast cells that were located in the edematous submucosa and between smooth muscle cells in the ileum of subjects with CD. In addition, evidence of degranulation of mast-cells, basophils and eosinophils in the affected area of ileum was also observed^[1]. Similarly, an increased number of mast-cells with intense degranulation was found in the active stage of ulcerative colitis (UC)^[3]. Moreover, it was also demonstrated that the median number of mast-cells in normal colonic tissue was significantly greater in patients with UC than controls (patients examined for colonic adenomas) or those with CD (1500 per milligram of tissue *vs* 1250 per milligram of tissue, $P < 0.05$)^[4]. Furthermore, median mast-cell counts obtained from inflamed colonic tissue were significantly ($P < 0.01$) greater than the number of mast-cells in non-inflamed tissue in patients with

IBD (2000 per milligram of tissue *vs* 1500 per milligram of tissue in UC and 1700 per milligram of tissue *vs* 1250 per milligram of tissue in CD)^[4]. On the other hand, King *et al*^[5] showed an increased mean number of mast cells (19.5) at the demarcation line between active and inactive areas of colonic inflammation in 12 of 20 (60%) UC patients. Finally, a Japanese group determined that patients with IBD or collagenous colitis had a greater number of mast cells in the upper part of the lamina propria of the colon than healthy controls and that patients with IBD had a higher number of mast cells in the lower part of the lamina propria of the colon as compared to those with collagenous colitis and healthy controls^[6].

Knuston *et al*^[7] observed that patients with CD of the distal ileum had a significantly greater mean histamine secretion rate within the small intestine than did healthy controls (152 ng/cm *vs* 71 ng/cm small intestine per hour, $P < 0.01$), and that histamine secretion was related to disease activity (active disease defined as CDAI > 150 : 193 ng/cm per hour *vs* inactive disease defined as CDAI < 150 : 105 ng/cm per hour). Further study also suggested that histamine secretion was significantly increased in inflamed colonic mucosa in patients with both CD and UC when compared to their non-inflamed colonic mucosa or colonic mucosa in healthy controls^[8]. A more recent study showed that urinary excretion of N-methylhistamine was significantly increased in patients with active IBD when compared to inactive IBD or non-IBD controls and such urinary histamine excretion strongly correlated with endoscopic activity of CD measured by the CD Endoscopic Index of Severity ($r^2 = 0.70$, $P < 0.0001$)^[9]. Greater expression of tumor necrosis factor- α (TNF- α) by mast cells was also found in the submucosa and muscularis propria of the ileum in patients with CD when compared to controls; significantly greater numbers of TNF- α -labeled mast cells were noted in the muscularis propria both in uninflamed (1.7-fold, $P < 0.05$) and in inflamed ileum (4.6-fold, $P < 0.002$)^[10]. In addition, TNF- α expression was found to be greater in the submucosa in inflamed *vs* uninflamed ileum in CD patients (1.6-fold, $P < 0.01$), while it was lower in the lamina propria in inflamed *vs* uninflamed ileum in CD patients (0.4-fold, $P < 0.05$)^[10]. This is noteworthy as TNF- α has been shown to be an important factor in the inflammatory cascade leading to the inflammatory response in the murine model for IBD^[11].

IgE

IgE as a biomarker of disease activity in IBD: It has been suggested that IgE may mediate an allergic response in patients with IBD. Evidence supporting this hypothesis includes the presence of peripheral and tissue eosinophilia in IBD patients^[12,13], increased numbers of mast cells or cells containing IgE in rectal mucosa of patients with IBD^[14,15], concomitant atopic disease in patients with IBD^[16,17] and a good response to disodium cromoglycate in IBD patients^[18-20].

Several studies have assessed IgE levels in patients with IBD. Pepys *et al*^[21] suggested that some patients with IBD (25% of UC patients and 31% of CD patients) may

have elevated serum IgE levels. These data were further supported by Levo *et al.*^[22], who claimed that patients with IBD have significantly increased mean serum level of IgE when compared to healthy controls (358 IU/mL *vs* 103 IU/mL, $P < 0.05$). On the other hand, several studies report normal serum IgE levels in IBD patients^[23-26]. Becker *et al.*^[25] determined that specific serum IgE levels to food allergens such as egg white, whole milk, β -lactoglobulin and wheat were undetectable in IBD patients and thus suggested that the allergic hypothesis of IBD should be rejected. However, a prior study by Mee *et al.*^[26] observed a significantly higher frequency of positive reactions to food allergens using the skin prick test in IBD patients when compared to healthy controls.

Role of IgE in desensitization to therapies for IBD:

Desensitization to sulfasalazine (SASP) has been found to be effective in patients who experienced hypersensitivity reactions to SASP^[27-30]. This is achieved by administration of successively larger doses of SASP, thereby allowing the presence of specific IgE in a controlled fashion without causing massive histamine release from mast cells^[29]. In the largest published study, reporting on 47 patients with IBD, desensitization was successful in 85% of patients with IBD, and there was no recurrence of hypersensitivity reactions in 82.5% of those who were successfully desensitized^[29]. In addition, among the successfully desensitized patients, 100% of UC patients and 78% of CD patients remained in long-term remission on SASP alone or in combination with intermittent prednisone^[29]. Caution is advised in attempting desensitization in patients with agranulocytosis, toxic epidermal necrolysis, or fibrosing alveolitis^[30]. The risks of these severe reactions may outweigh the benefit of continuing to take SASP containing medications.

PROBIOTICS

At a workshop held at Yale University in 2007, recommendations were made with regards to the use of probiotics in clinical settings for a variety of indications, including IBD^[31]. For IBD, the recommendations included a class “A” recommendation (defined as one made on “strong, positive, well-conducted controlled” studies) for the use of VSL#3 for the prevention and maintenance of remission in pouchitis^[31]. In addition, some probiotics were given a class “C” recommendation (one “based on some positive studies”) for IBD^[31]. These included VSL#3 for the induction of remission of pouchitis, and for inducing remission and maintenance of remission in ulcerative colitis^[31]. The probiotic *Lactobacillus* GG (LGG) was noted to be beneficial in both IBD and allergic diseases; LGG has a “C” class recommendation in the treatment of CD, and a class “A” designation treatment of atopic eczema^[31]. Probiotics may also help with cow milk allergy^[31]. See Tables 1-3 for other disease indications and probiotic regimens, with additional recommendations from the workshop in 2007.

Lactobacillus also has specific bacteriocidal effects, including against *Lactococcus*, *Streptococcus*, *Staphylococcus*, *Listeria* and *Mycobacteria*^[32]. The bacterium uses two different pathways to neutralize competing bacteria. First, it uses a

Table 1 Recommendations for probiotic use

Clinical condition	Effectiveness	Organism
Diarrhea		
Infectious-adult-treatment	A	<i>Saccharomyces boulardii</i> , LGG
Infectious-childhood-treatment	A	LGG, <i>Lactobacillus reuteri</i>
Prevention of infection	B	<i>S. boulardii</i> , LGG
Prevention of AAD	A	<i>S. boulardii</i> , LGG, <i>L. casei</i> , <i>L. bulgaricus</i> , <i>S. thermophilus</i>
Treatment of recurrent CDAD	B	<i>S. boulardii</i> , LGG
Prevention of CDAD	B	LGG, <i>S. boulardii</i>
IBD		
Pouchitis		
Preventing and maintaining remission	A	VSL#3
Induce remission	C	VSL#3
Ulcerative colitis		
Inducing remission	C	<i>Escherichia coli</i> Nissle, VSL#3
Maintenance	C	<i>E. coli</i> Nissle, VSL#3
Crohn's	C	<i>E. coli</i> Nissle, <i>S. boulardii</i> , LGG
IBS	B	<i>Bifidobacterium infantis</i>
IBS	C	<i>Bifidobacterium animalis</i> , VSL#3, <i>Lactobacillus plantarum</i>
Immune response	A	LGG, <i>Lactobacillus acidophilus</i> , <i>L. plantarum</i> , <i>Bifidobacterium lactis</i> , <i>Lactobacillus johnsonii</i>
Allergy		
Atopic eczema assoc. with cow milk allergy		
Treatment	A	LGG, <i>B. lactis</i>
Prevention	A	LGG, <i>B. lactis</i>
Radiation enteritis	C	VSL#3, <i>L. acidophilus</i>
Vaginosis and vaginitis	C	<i>L. acidophilus</i> , LGG, <i>L. reuteri</i>

Reproduced with permission from reference Floch *et al.*^[31]. An “A” recommendation is based on strong, positive, well-conducted, controlled studies in the primary literature, not abstract form; A “B” recommendation is based on positive, controlled studies but the presence of some negative studies; A “C” recommendation is based on some positive studies. IBD: Inflammatory bowel disease; LGG: *Lactobacillus* GG; *S. boulardii*: *Saccharomyces boulardii*.

small molecule to inhibit growth of competitor bacteria, likely a short chain fatty acid, and also uses hydrogen peroxide to alter the bacterial microenvironment from an aerobic one to an anerobic one^[32]. In addition, *Lactobacillus* also has immunomodulatory abilities. First, the bacillus increases transepithelial resistance, and it also upregulates the key toll-like receptors TLR2 and TLR9. These toll-like receptors recognize foreign hostile viral and bacterial antigens and their activation causes a large expansion of cytokine expression and amplifies the immune response against hostile antigens^[32].

ATOPY, NASAL DISEASE AND URTICARIA IN IBD

Prior studies have described a relationship between IBD

Table 2 Results of random controlled trials which reported efficacy of probiotics in patients with inflammatory bowel disease

Probiotic	Situation	Control	No. of subjects	Duration (mo)	Relapse probiotic vs control	Ref.
<i>E. coli</i> Nissle 1917	Ulcerative colitis	5-ASA	120	4	16% vs 11.3%	[70]
<i>E. coli</i> Nissle 1917	Ulcerative colitis	5-ASA	120	12	67% vs 73%	[71]
<i>E. coli</i> Nissle 1917	Crohn's disease	Placebo	28	12	30% vs 70% ^a	[72]
VSL#3	Pouchitis	Placebo	40	9	15% vs 100% ^a	[73]
VSL#3	Prevention of pouchitis	Placebo	40	12	10% vs 40% ^a	[74]
VSL#3	Crohn's disease ¹	5-ASA	28	12	20% vs 40% ^a	[75]
<i>S. boulardii</i>	Ulcerative colitis	5-ASA	31	12	30% vs 35% ^a	[76]
<i>S. boulardii</i>	Crohn's disease	5-ASA	28	6	6.3% vs 37.5% ^a	[77]

Reproduced with permission from reference Marteau^[69]. ¹Postoperative. ^a*P* < 0.05 vs control. 5-ASA: 5-aminosalicylic acid; *E. coli*: *Escherichia coli*; *S. boulardii*: *Saccharomyces boulardii*.

Table 3 Probiotics in treatment of inflammatory bowel disease: Randomized controlled trials

Entity	Ref.	Population (n)	Probiotic(s)	Treatment group	Effect
Crohn's disease	Bousvaros <i>et al</i> ^[79]	Children (75; age, 5-21 yr)	LGG	Maintenance of remission	No benefit
	Plein <i>et al</i> ^[80]	Adults (20)	<i>S. boulardii</i>	Maintenance of remission	Reduced diarrhea vs placebo
	Malchow ^[72]	Adults (28)	<i>E. coli</i> Nissle 1917	Maintenance of remission	No benefit
	Prantera <i>et al</i> ^[81]	Adults (45)	LGG	Maintenance of surgically induced remission	No benefit
Ulcerative colitis	Schultz <i>et al</i> ^[82]	Adults (11)	LGG	Treatment of active Crohn's disease	No benefit
	Kruis <i>et al</i> ^[70]	Adults (120)	<i>E. coli</i> Nissle 1917	Maintenance of medically induced remission	Equal to mesalamine
	Rembacken <i>et al</i> ^[71]	Adults (120)	<i>E. coli</i> Nissle 1917	Maintenance of medically induced remission	Equal to mesalamine
	Kruis <i>et al</i> ^[83]	Adults (327)	<i>E. coli</i> Nissle 1917	Maintenance of medically induced remission	Equal to mesalamine
Pouchitis	Ishikawa <i>et al</i> ^[84]	Adults (21)	<i>B. breve</i> and <i>B. bifidum</i> and <i>L. acidophilus</i> YIT 0168	Maintenance of medically induced remission	Superior to placebo
	Gionchetti <i>et al</i> ^[73]	Adults (40)	VSL#3	Maintenance of antibiotic-induced remission of chronic pouchitis	Superior to placebo
	Mimura <i>et al</i> ^[85]	Adults (36)	VSL#3	Maintenance of antibiotic-induced remission of chronic pouchitis	Superior to placebo
	Gionchetti <i>et al</i> ^[86]	Adults (40)	VSL#3	Maintenance of antibiotic-induced remission of chronic pouchitis	Superior to placebo
	Kuisma <i>et al</i> ^[87]	Adults (20)	LGG	Treatment of active pouchitis	No benefit

Reproduced with permission from reference Szajewska *et al*^[78]. LGG: *Lactobacillus GG*; *E. coli*: *Escherichia coli*; *S. boulardii*: *Saccharomyces boulardii*; *B. breve*: *Bifidobacterium Breve*; *B. bifidum*: *Bifidobacterium bifidum*; *L. acidophilus*: *Lactobacillus acidophilus*.

and allergy. It is unclear whether this association is manifested as atopy (consisting of atopic dermatitis, allergic rhinitis, asthma, and food allergy) or nasal disease. The association was hypothesized because patients with allergy have an abnormal IgE antibody response to common environmental antigens and earlier findings of peripheral and tissue eosinophilia in patients with IBD had suggested an IgE mediated response. Furthermore, because some allergic symptoms were associated with other systemic inflammatory disorders, it was felt that patients with colitis might also have an increased likelihood of developing atopic illnesses.

A review of prior studies has shown that these results may be equivocal and may only be pertinent in relation to food allergies and possibly sinonasal disease. One study showed that in children with atopic eczema, food allergy is associated with increased intestinal inflammation, as manifested by elevated levels of fecal eosinophil cationic protein, TNF- α and α 1 antitrypsin^[33]. Similarly, in another study, while there was no correlation observed between

frequency of personal history of atopy, serum IgE levels and prick test response between IBD patients and controls, it was observed that IBD patients had a higher frequency of positive prick test when tested against food allergens^[26]. There also seemed to be a positive relationship between IBD and chronic sinonasal disease, since the prevalence of chronic sinonasal disease was elevated in patients with IBD, specifically in patients with CD and especially in those also with obstructive bowel complications^[34]. Interestingly, nearly 70% of these patients had some degree of sinonasal disease; see Figure 1 for a graph depicting the prevalence of sinonasal disease in patients with IBD.

Other atopic features (asthma, hay fever and allergic rhinitis) were investigated in patients with UC and CD with the results indicating that atopic features were twice as common in patients with UC, but no different between patients with CD and controls, suggesting that hypersensitivity may play a part in UC but not in CD^[17].

In contrast, other studies found no association be-

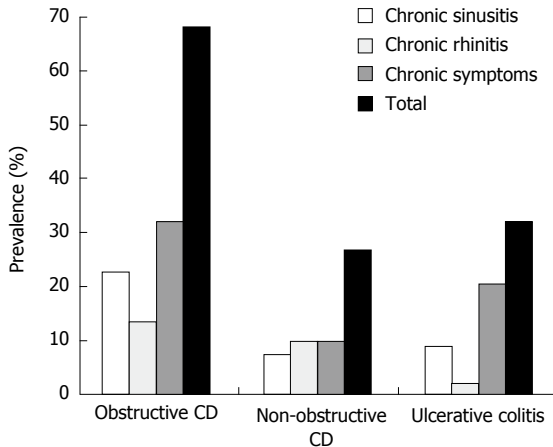


Figure 1 Prevalence of sinonasal disease in inflammatory bowel disease patients. Reproduced with permission from reference Book *et al*^[34]. CD: Crohn's disease.

tween controls and IBD patients in terms of atopic symptoms. Personal history analyses for atopic symptoms (asthma, allergic rhinitis, eczema, urticaria, and allergic symptoms) were assessed in another study where the prevalence of atopy with skin-prick tests (using five allergens - mixed grass pollens, dog hair, cat fur, *Dermatophagoides pteronyssinus* and *Aspergillus fumigatus*) and serum IgE concentrations among 122 patients with IBD and 103 age-matched controls was examined^[35]. The authors showed no statistical difference in the percentage of patients with positive skin tests between controls and IBD patients and also no difference between the subgroups of IBD patients (CD, ulcerative colitis, and ulcerative proctitis)^[35].

Another study assessed skin test reactivity in IBD patients. In this study, two different populations were examined, one in the United States and another in Czechoslovakia, and patients were classified as having CD or UC by clinical, pathological and radiological criteria; those who had been treated with immunosuppressives were taken off these medications for three weeks. The authors found no evidence of skin test anergy (assessed by using multiple antigens - candida, mumps, PPD, streptokinase-streptodornase, and trichophyton) when all patients were compared to controls^[36].

Despite initial studies that suggested an association between IBD and IgE mediated allergic reactions, direct evidence is still lacking and the role of IgE mediated reactions remains unclear.

NUTRITION AND IBD

Epidemiology of food intolerance and IBD

IBD is characterized by chronic inflammation of the gastrointestinal tract. The etiology of IBD is complex and probably multi-factorial. Nutrition is an important modulator of IBD^[37]. In particular, it is felt that relationships between food antigens and immune pathways may alter the course of IBD^[38]. Gut bacteria and the inflammatory response are altered by the ingestion of differing foods^[39].

Most patients with IBD are intolerant to selected (or

specific) food items. Food intolerance has been evaluated in multiple studies. In a survey administered to 132 patients (along with 70 controls) with IBD, food intolerance was reported by 66% of patients with CD, and 64% of UC patients^[40]. The most common symptoms included diarrhea and abdominal pain^[40]. In a study that evaluated the antibody response to cow's milk antigen, it was found that IgG and IgM antibodies to beta-lactoglobulin were significantly elevated in patients with UC and CD when compared with normal subjects^[41]. Elevation of IgG further correlated with involvement of the ileum, increase in inflammatory markers, and was higher in untreated patients; interestingly there was no change in IgM levels after sulfasalazine or steroid therapy^[41]. Another study reported on a questionnaire examining dietary habits, the amount of food consumed, and whether patients had problems with specific foods^[42]. A total of 122 foods were evaluated. Intolerance to chocolate, dairy products, fats and artificial sweeteners were seen in both UC and CD, and patients with CD seemed to have a greater range of problems with specific foods^[42]. From 80%-100% of bacteria in the colonic flora of CD patients are bound by immunoglobulin whereas, in controls only 20% are bound; when enteral feeds are given the percentage bound in CD significantly decreases^[43,44].

The prevalence of food reactions was studied in 375 adult patients attending a gastroenterology outpatient clinic: 32% complained of food allergies as being the source of their abdominal complaints and in 14.4% laboratory testing was consistent with intestinal food allergy^[45]. Laboratory testing included counts of eosinophils, the presence of specific IgE against food antigens, increased total IgE, specific clinical signs of atopy, and cromoglycate sensitivity^[45]. Confirmation of the diagnosis of food allergy was found in 3.2%^[45]. This was confirmed through elimination diet and subsequent rechallenge or allergen provocation testing during EGD^[45].

Breastfeeding may have a protective effect against developing IBD^[37,46]. Of thirteen reported case-control studies, 3/13 (23.1%) found that patients with IBD were less likely to have been breast fed as compared to controls^[37]. In another study of 308 matched patients, the patients with CD were found to have had an average breast-feeding duration of 4.6 mo as compared to controls who had an average duration of 5.8 mo^[47]. Postulated mechanisms have been suggested to include a protective effect of immunoglobulins and antibacterial proteins in breast milk^[37]. In addition, breast milk may accelerate maturation of the GI tract in infants, and may also delay the introduction of cow's milk, a potentially allergenic food^[37]. Another population based case-control study examined three cohorts of patients: one with 638 CD patients, one with 653 UC patients, and 600 controls^[46]. Specific factors associated with a lower odds ratio of CD and UC included breast-feeding (CD: OR = 0.55; 95%CI: 0.41-0.74; UC: OR = 0.71; 95%CI: 0.52-0.96), and having a vegetable garden during infancy, childhood or adolescence (CD: OR = 0.52; 95%CI: 0.36-0.76; UC: OR = 0.65; 95%CI: 0.45-0.94)^[46]. In addition, those living in the countryside had a lower odds ratio of having CD (OR = 0.64; 95%CI: 0.46-0.89).

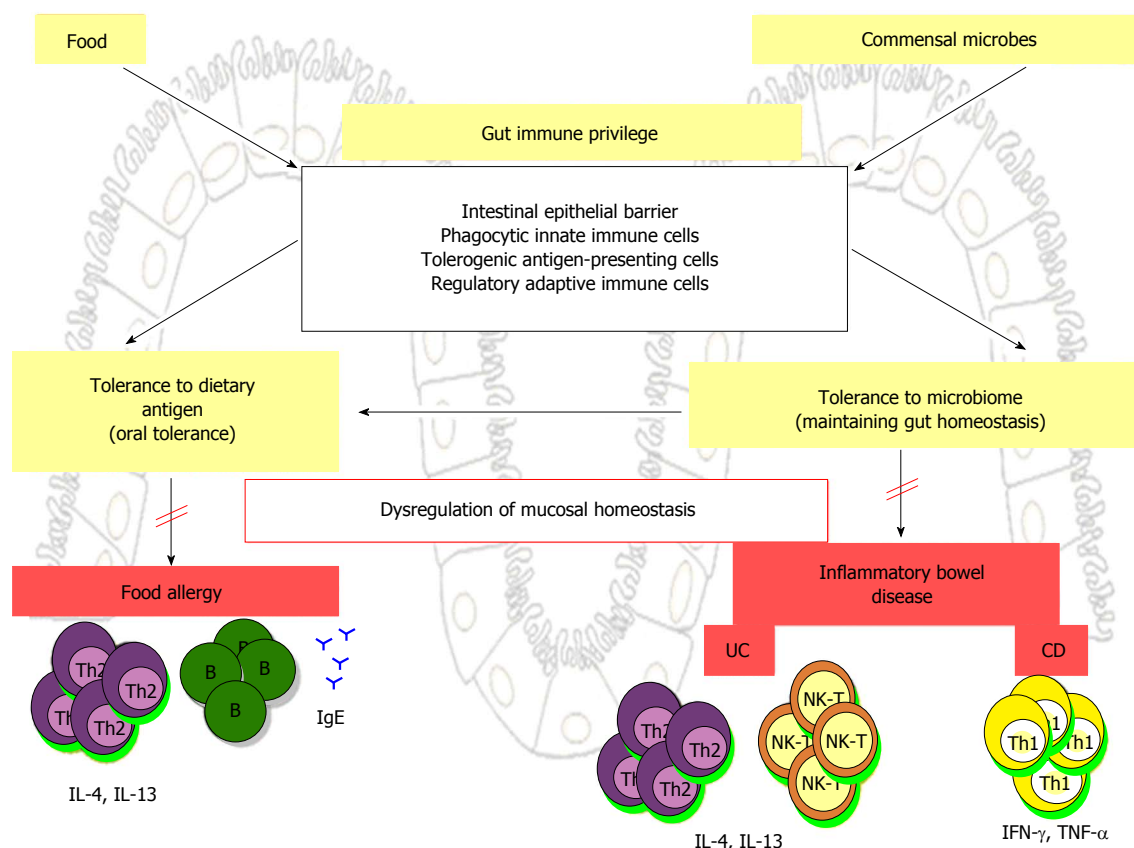


Figure 2 Immune privilege in the gut consists of tolerance to dietary antigens and to commensal microbes^[49]. Reproduced with permission from reference Iweala *et al*^[49]. CD: Crohn's disease; UC: Ulcerative colitis; TNF-α: Tumor necrosis factor-α; IL: Interleukin; IFN: Interferon.

The duration of breastfeeding was also significant in decreasing both IBD and UC, with those having 0-2 mo of exposure having no protection, as compared with those having 3 or more mo^[46].

Relation between pathophysiology of food insensitivity and IBD

While 20%-30% of the general population have undesirable reactions to food, only 10%-25% of these are actual food allergies^[48]. The gastrointestinal mucosa is however predisposed to develop allergic reactions, since the tissue in the GI tract is exposed to various food and bacterial allergens in addition to containing all cells required to develop allergic reactions, such as eosinophils, mast cells, and lymphocytes^[48].

Proteins in food usually act as the primary foreign antigens that trigger most allergic reactions in the gastrointestinal tract^[49]. The border between intra and extraluminal sites in the intestine plays a vital role in preventing inappropriate inflammation or allergic disease in the gut^[49,50]. The gut barrier works in at least five different ways to prevent such diseases. These include (Figure 2): (1) a physical barrier preventing foreign or microbial invasion; (2) the presence of specialized immune cells, including macrophages, to phagocytize microbes and other proinflammatory or allergic antigens; (3) release of IgA, which sequesters microbes away from the gut in the intraluminal space; (4) promotion of antigen presenting cells which upregulate immune tolerance; and (5) expansion of regu-

latory T cells which dampens the immune response^[49]. Chronic inflammation in the gut may act in an ongoing fashion with increasing inflammation leading to additional damage to the physical barrier of the gut, leading in turn to more proinflammatory antigens which can then pass into the extraluminal space^[51].

A disruption of one of these five regulatory barriers may contribute to inflammation and/or allergic responses. Mast cells and eosinophils also play a critical role in modulating intestinal allergic reactions and have also been found to be stimulated in IBD and eosinophilic gastroenteritis^[52].

In addition, the main inflammatory mechanisms of IBD and of the pathophysiology of intestinal allergic phenomena are related concepts. In particular, the Th2-like response seen in ulcerative colitis is driven by NK-T cells activated by glycolipid and CD1 on epithelial cells, which facilitates their production of proinflammatory cytokines including IL-13 and IL-5^[53]. Interestingly, this process does not result in the production of IL-4. Upregulation and maladaptive Th2 responses have been implicated in chronic rhinosinusitis^[54]. In addition, *Staphylococcus aureus* (*S. aureus*), a common colonizer of the nasal tract, has been shown to upregulate IL-5 and IL-13 in nasopharyngeal lymphocytes^[54].

Additionally, there is also maladaptive activity of T regulatory T cells (Tregs) both in IBD and in food intolerance^[49]. Adoptive transfer of Tregs has been shown to prevent intolerance to the food antigen OVA^[55]. In addition it has been found that TLR4 signaling is critical for

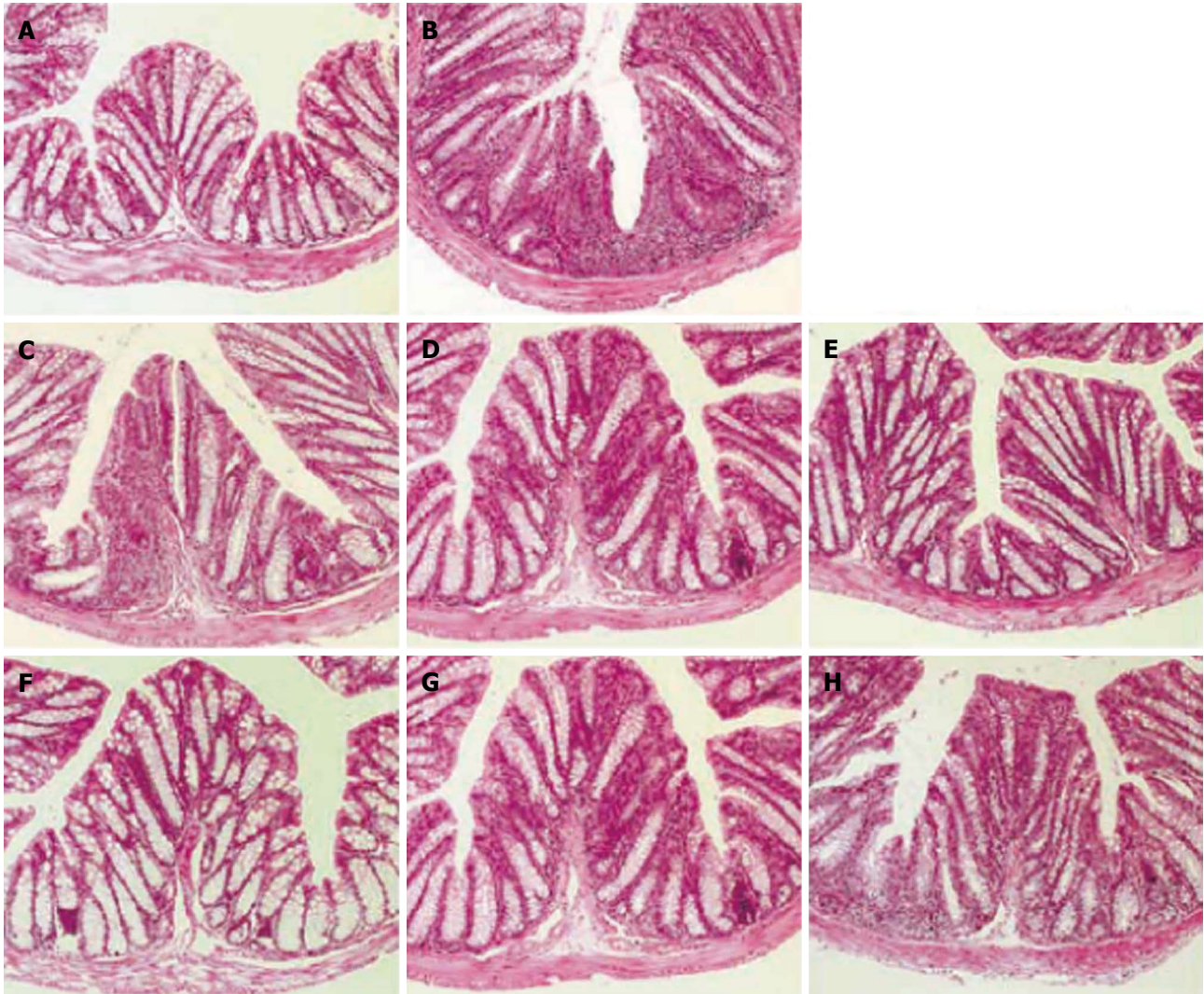


Figure 3 Histologic analysis of the colon in C57BL/6 mice. A: Normal architecture of the colonic mucosa from mice treated with 50% ethanol alone; B: Erosions of the epithelium, distortion of crypts, loss of goblet cells, and massive mononuclear cell infiltration in lamina propria in mice after administration of trinitrobenzene (TNBS); C-E: TNBS-induced colitis is dose-dependently improved by curcumin. Mice were treated with 0.5% (C), 2.0% (D), or 5.0% (E) curcumin just after administration of TNBS; F-H: Mice were treated with 2% curcumin in preventive mode (F), early therapeutic mode (G), or late therapeutic mode (H) (original magnification $\times 50$). Reproduced with permission from reference Sugimoto *et al*^[66].

Treg suppression of responses to food antigens (regulating food tolerance) and also to commensal bacteria (in prevention of colitis)^[49].

Common treatments for food insensitivity and IBD

Alterations in diet have been used as a treatment for IBD. The use of enteral feeding has been shown to be beneficial, with a majority of studies showing a remission rate of over 60%^[56]. Liquid feeding is thought to be helpful because it leads to more rapid transit time for feeds, induces partial bowel rest, as well as alters the fecal flora present^[57]. Tube feeding with an enteral elemental liquid formula cannot be used long-term however, as most patients do not tolerate this, and IBD typically relapses after discontinuation of the diet^[57]. However, the use of polymeric drinks may be better tolerated and shows similar effectiveness in this population^[57].

The use of elimination diets has also been studied. These typically involve the use of a food diary, with elimi-

nation of symptom provoking foods, or the use of a basic diet with reintroduction of potentially problematic foods one food type at a time^[58]. This approach was shown to be beneficial in inducing remission in six of nine studies^[58]. Referral to a nutritionist may be of significant benefit^[58].

Other potential therapies in the treatment of IBD include the use of polyunsaturated fatty acids (PUFA). These acids, including omega-3 fatty acids, decrease inflammation as their derivatives, including eicosapentaenoic acid, and leukotriene derivatives, downregulate neutrophil trafficking and thereby decrease edema formation^[59-61]. Twelve studies with n-3 PUFA, mostly from fish oil, showed benefit in IBD patients, with a decreased need for steroids, diminished disease activity and a lower relapse rate^[59].

Several herbal preparations are considered useful by some in the treatment of inflammatory disorders. *Lonicera japonica* is a Korean traditional treatment, and has been shown to decrease histamine release from mast

cells and inhibit inflammatory pathways, including the NF- κ B and AP-1 pathways^[62-64]. Lonicera may be an attractive agent for future clinical trials in both IBD and allergic disease^[62]. In addition, curcumin and green tea polyphenols have been shown to be potent antioxidants and have anti-inflammatory activity in mice^[65-67]. In one study, 35.5% of mice treated with trinitrobenzene (TNBS) died after developing an ulcerative colitis-like disease; however, no mice died in a group preventively given a 2% curcumin solution^[66]. In addition, mice given doses of curcumin after TNBS-induced colitis demonstrated histologic improvement of colonic mucosa^[66] (Figure 3).

DIFFERENCES BETWEEN EOSINOPHILIC COLITIS AND IBD

Eosinophilic gastrointestinal disorder (EGID) is marked by GI inflammation and intense infiltration of the GI tract with eosinophils seen in the absence of other identified systemic disorders such as malignancy, collagen-vascular disease, IBD, parasitic disease, and medication induced eosinophilia^[68]. In 50%-70% of patients with EGID, there is a family history of allergic disorders or a personal history of atopy^[48]. Symptoms vary between children and adults: in children, the common symptoms are vomiting and abdominal pain, while in adults common symptoms include food impaction, difficulty swallowing, chest pain and heartburn^[68]. Symptoms mimic both irritable bowel syndrome and IBD. Histologically, EGID can be distinguished from GERD; more than 15-20 eosinophils per high power field are seen on biopsy in EGID as opposed to less than 5 in GERD^[48,68]. Both elimination and elemental diets have shown promise in the treatment of patients with EGID^[48,68].

CONCLUSION

While the immunological underpinnings of both IBD and allergic disease are complex and multifaceted, the degree of overlap between the two disorders is striking. Further studies are warranted to try to help better understand their complex basis and commonality, and there is much to be gained by studying treatments that benefit patients with these illnesses.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Small bowel adenocarcinoma and Crohn's disease: Any further ahead than 50 years ago?

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bowel disease

Core tip: This review of the literature on small bowel carcinoma associated with Crohn's disease specifically addresses the incidence, risk factors, and protective factors which have been identified. It also reviews the clinical presentation, the current modalities of diagnosis, the pathology, treatment, and surveillance. Finally, the prognosis and future direction are addressed. Our experience with small bowel adenocarcinoma in Crohn's disease is reported. Readers will be provided with a better understanding of this rare and often poorly recognized complication of Crohn's disease.

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Abstract

This review of the literature on small bowel carcinoma associated with Crohn's disease specifically addresses the incidence, risk factors, and protective factors which have been identified. It also reviews the clinical presentation, the current modalities of diagnosis, the pathology, treatment, and surveillance. Finally, the prognosis and future direction are addressed. Our experience with small bowel adenocarcinoma in Crohn's disease is reported. Readers will be provided with a better understanding of this rare and often poorly recognized complication of Crohn's disease.

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Key words: Crohn's disease; Small bowel adenocarcinoma; Cancer risk; Cancer malignancy; Incidental carcinoma; Late complications of Crohn's disease; Inflammatory

INTRODUCTION

Over the past several decades it has become increasingly recognized that small bowel adenocarcinoma is an undeniable complication of Crohn's disease of the small intestine. The exact magnitude of this risk is virtually impossible to determine. The first case of small bowel carcinoma in Crohn's disease was reported by Ginzburg in 1956. Since then, there have been countless published case reports as well as numerous retrospective reviews and cohort studies which have attempted to define the occurrence of small bowel carcinoma in Crohn's disease.

This review of the literature on small bowel carcinoma associated with Crohn's disease specifically addresses the incidence, risk factors, and protective factors which have been identified. It will also review the clinical pre-

sentation, the current modalities of diagnosis, the pathology, treatment, and surveillance. Finally, the prognosis and future direction will be addressed. Our experience with small bowel adenocarcinoma in Crohn's disease is reported. Readers will be provided with a better understanding of this rare and often poorly recognized complication of Crohn's disease.

INCIDENCE

The relative risk of developing carcinoma of the small bowel in patients with Crohn's disease has been estimated to range from 6 to 320^[1]. Jess *et al*^[2] studied a population-based cohort of 374 patients with Crohn's disease in order to determine the long term risk of intestinal and extra-intestinal malignancies. The risk of small bowel adenocarcinoma was increased by more than 60 fold as compared to the general population. On the basis of 5000 cases of Crohn's disease reported in the world literature, Amman^[3] calculated an association with carcinoma of 0.08%, which, he emphasized, was lower than the incidence of carcinoma of the small bowel in the absence of Crohn's disease, which he estimated at 0.098% in 137124 autopsies. However, the incidence in Crohn's disease may be higher than reported as the associated carcinoma may have been missed due to inadequate histopathologic study. These observational series serve to underline the possible fallacies of mass statistics collected from the literature.

Our review of the literature revealed 220 reported cases of adenocarcinoma associated with small bowel Crohn's disease. Since we do not have the number of patients who suffered from Crohn's disease of the small bowel from which those patients were extracted, the actual incidence of carcinoma associated with Crohn's cannot be calculated. These numbers were obtained through a Medline search from the years 1975 to 2013. Some of the reports were reviews and hence there may be some duplication of cases in the series reported.

There is nevertheless a general consensus that the risk of developing small bowel adenocarcinoma is greater in patients with Crohn's disease than in the general population. The exact magnitude of the increased risk is difficult to determine because information derived from population-based studies, case-controlled studies, physician surveys, and case reports have been combined.

Many reports have documented that adenocarcinoma of the small bowel is a complication of Crohn's disease and this has been well reviewed by Kerber^[4] and Frank^[5]. Sometimes the patients who develop an adenocarcinoma are those with small bowel-limited Crohn's disease but most often there is a combination of those with both small and large bowel Crohn's disease. Patients with longstanding small bowel Crohn's disease are thought to have an increased risk of small bowel carcinoma^[6-9].

In a meta-analysis by von Roon *et al*^[10], the relative risk of developing small bowel carcinoma in the 9642 patients was 28.37. The incidence rate of small bowel carcinoma

in 12740 patients was 1.55 per 100000 patient years. In the review by Von Roon, the mean duration of Crohn's disease before the onset of carcinoma was 9 years (range 0.8-41). The relative risk of developing small bowel carcinoma compared to the background population was higher in North America (RR = 41.23), the United Kingdom (RR = 40) and Scandinavia (RR = 21.3).

Shaukat *et al*^[11] studied all cases of small bowel carcinoma in persons 67 years and older in the Surveillance Epidemiology and End Results catchment area. They identified 923 cases of small bowel carcinoma and 142273 controls and found a strong association between Crohn's disease and small bowel carcinoma [odds ratio (OR) = 12.02]. The prevalence of Crohn's disease in patients with small bowel carcinoma was low (1.6%) so the absolute risk remains low.

RISK AND PROTECTIVE FACTORS

Numerous risk factors for developing small bowel carcinoma in Crohn's disease have been postulated in the literature. Many purported risk factors surfaced as a result of observed trends across case reports such as previous stricturoplasty^[12-15] and excluded/bypassed bowel segments^[16-18]. Approximately 30% of reported small bowel carcinomas in Crohn's disease occurred in patients who had bypassed loops^[19]. This complication stresses the need to encourage resection rather than bypass. Lashner^[20] reported a case control study of carcinoma of the small bowel in Crohn's disease in which each case was matched to 4 randomly selected controls from an inflammatory bowel disease registry. The following factors were significantly associated with small bowel carcinoma in Crohn's disease: (1) occupation, with three cases having had exposure to halogenated aromatic compounds and aliphatic amines, asbestos, and cutting oil solvents and abrasives; and (2) 6-mercaptopurine use (OR = 10.8).

Several studies reported that the risk of developing small bowel carcinoma was according to the anatomic location of the Crohn's disease (Table 1). For example, the odds ratio of developing small bowel carcinoma was found to be much higher in 363 patients whose disease was confined to the small bowel (RR = 158.5) than in the 507 patients with ileocolic Crohn's disease (RR = 83.8)^[10].

Table 1 displays a review of the studied risk factors for small bowel carcinoma in Crohn's disease in the literature, highlighting which authors agree and disagree with a purported risk factor. Clinicians should perhaps be more vigilant for small bowel carcinoma in patients with inflammation restricted to the small bowel versus ileocolic inflammation.

Protective factors against development of small bowel carcinoma in Crohn's disease have been less frequently studied, but available information is cited in Table 2. In a study of 29 patients with Crohn's disease and small bowel carcinoma Piton *et al*^[21] found that small bowel resection and prolonged use of salicylates may protect against small bowel carcinoma in Crohn's disease patients.

Table 1 Risk factors for small bowel cancer in Crohn's disease

Risk factors for small bowel cancer?		
	Yes	No
Crohn's related risk factors		
1 "Long" duration of CD	Greenstein <i>et al</i> ^[68] (mean 33.5 yrs) Greenstein <i>et al</i> ^[24] (mean 22 yrs) Jess <i>et al</i> ^[21] (median 16 yrs) Kamiya <i>et al</i> ^[69] (one case 18 yrs) Kersting <i>et al</i> ^[65] (one case 16 yrs) Kvist <i>et al</i> ^[70] (mean 29 yrs) Mellemkjaer <i>et al</i> ^[71] (mean 22 yrs) Michelassi <i>et al</i> ^[55] (mean 19.6 yrs) Mizushima <i>et al</i> ^[72] (mean 14 yrs) Munkholm <i>et al</i> ^[8] (mean 13.5 yrs) Palascak-Juif <i>et al</i> ^[27] (median 15 yrs) Petras <i>et al</i> ^[46] (mean 20 yrs) Ribeiro <i>et al</i> ^[11] (mean 26.5 yrs) Savoca <i>et al</i> ^[62] (one case 20 yrs) Sigel <i>et al</i> ^[48] (median 12 yrs) Solem <i>et al</i> ^[26] (median 21 yrs) Widmar <i>et al</i> ^[23] (mean 25.3 yrs)	Jess <i>et al</i> ^[73] Laukoetter <i>et al</i> ^[63]
2 Area of CD inflammation	Jess <i>et al</i> ^[21] Michelassi <i>et al</i> ^[55] Mizushima <i>et al</i> ^[72] Munkholm <i>et al</i> ^[8] Palascak-Juif <i>et al</i> ^[27] Ribeiro <i>et al</i> ^[11] Savoca <i>et al</i> ^[62] Solem <i>et al</i> ^[26] von Roon <i>et al</i> ^[10]	Laukoetter <i>et al</i> ^[63]
3 Jejunal CD	Lashner ^[20]	Palascak-Juif <i>et al</i> ^[27] Solem <i>et al</i> ^[26] Solem <i>et al</i> ^[26]
4 Strictures	Jaskowiak <i>et al</i> ^[15] Kersting <i>et al</i> ^[65] Lakatos <i>et al</i> ^[74] Petras <i>et al</i> ^[46] Ribeiro <i>et al</i> ^[11]	Lashner <i>et al</i> ^[20] Solem <i>et al</i> ^[26] Lashner ^[20] Palascak-Juif <i>et al</i> ^[27] Ribeiro <i>et al</i> ^[11] Solem <i>et al</i> ^[26]
5 Fistula	Kersting <i>et al</i> ^[65] Laukoetter <i>et al</i> ^[63] Ribeiro <i>et al</i> ^[11]	Lashner <i>et al</i> ^[20] Solem <i>et al</i> ^[26] Lashner ^[20]
6 Bypassed segment	Greenstein <i>et al</i> ^[75]	Palascak-Juif <i>et al</i> ^[27] Ribeiro <i>et al</i> ^[11] Solem <i>et al</i> ^[26]
7 CD Medications	¹ Lashner ^[20]	Canavan <i>et al</i> ^[76] Solem <i>et al</i> ^[26]
8 "Young" age	Freeman <i>et al</i> ^[60] (mean 45.7 yrs) Hoffman <i>et al</i> ^[7] (mean 46 yrs) Kersting <i>et al</i> ^[65] (one case 34 yrs) Laukoetter <i>et al</i> ^[63] (20 yrs earlier) Michelassi <i>et al</i> ^[55] (mean 47.7 yrs) Palascak-Juif <i>et al</i> ^[27] (median 47 yrs) Petras <i>et al</i> ^[46] (mean 46 yrs) Savoca <i>et al</i> ^[62] (mean 38 yrs) Sigel <i>et al</i> ^[48] (median 42 yrs) Widmar <i>et al</i> ^[23] (mean 55.4 yrs)	Jess <i>et al</i> ^[73] (median 66 yrs) Munkholm <i>et al</i> ^[8] (mean 70.5 yrs)

General risk factors

9 Gender		Jess <i>et al</i> ^[21] Petras <i>et al</i> ^[46] Lashner ^[20] Palascak-Juif <i>et al</i> ^[27]
10 Male	Lakatos <i>et al</i> ^[74] Michelassi <i>et al</i> ^[55] Shaukat <i>et al</i> ^[111] Sigel <i>et al</i> ^[48] Ribeiro <i>et al</i> ^[11] Widmar <i>et al</i> ^[23] Freeman <i>et al</i> ^[60] Shaukat <i>et al</i> ^[111]	
11 Female		
12 Black race		
13 Past corticosteroid use		Kaerlev <i>et al</i> ^[67]
14 Past use of radioactive medication		Kaerlev <i>et al</i> ^[67]
15 Liver disease (cirrhosis/hepatitis)		Kaerlev <i>et al</i> ^[67]
16 Gallstones		Kaerlev <i>et al</i> ^[67]
17 Previous cholecystectomy	Chen <i>et al</i> ^[64]	Kaerlev <i>et al</i> ^[67]
18 Prior history of peptic ulcer disease	Chen <i>et al</i> ^[64]	Kaerlev <i>et al</i> ^[67]
19 Celiac disease	Kaerlev <i>et al</i> ^[67]	
20 Prior malignancy		Solem <i>et al</i> ^[26]
21 Blood type B		Chen <i>et al</i> ^[64]
22 Rh type		Chen <i>et al</i> ^[64]
23 Tobacco	Chen <i>et al</i> ^[64] Lakatos <i>et al</i> ^[74] Chen <i>et al</i> ^[64]	Chow <i>et al</i> ^[77] Negri <i>et al</i> ^[25] Chow <i>et al</i> ^[77] Negri <i>et al</i> ^[25]
24 Alcohol		
25 Diet	² Negri <i>et al</i> ^[25] ³ Chow <i>et al</i> ^[77] Kaerlev <i>et al</i> ^[67]	
26 Lower education level	Kaerlev <i>et al</i> ^[67]	
27 Geographic location	⁴ von Roon <i>et al</i> ^[10]	
28 Hazardous occupation	⁵ Lashner ^[20]	
29 Marital status		Chen <i>et al</i> ^[64]
30 Religion		Chen <i>et al</i> ^[64]
31 Room type		Chen <i>et al</i> ^[64]

¹6-MP; ²Bread, pasta, rice, sugar, red meat; ³Red meat, salt-cured foods, smoked foods; ⁴North America higher risk; ⁵Exposure to halogenated aromatic compounds and aliphatic amines, asbestos, solvents, oils, abrasives. CD: Crohn's disease.

CLINICAL PRESENTATION

Obstruction is the most common presenting manifestation in small bowel carcinoma in Crohn's disease with symptoms of nausea, vomiting and abdominal pain. Other possible presentations are hemorrhage, fistula, or perforation^[22-24]. Unfortunately, all of these symptoms are hard to differentiate from those of a Crohn's exacerbation, which partly explains the challenge of detecting small bowel carcinoma in this patient population and results in the majority of diagnoses being made at the time of operation or postoperatively. In fact, only a small minority (< 5%) is diagnosed preoperatively^[22]. Furthermore, Collier *et al*^[19] described that over 50% of small bowel carcinomas in resected Crohn's disease segments were unsuspected or incidentally found by the pathologist.

Table 2 Protective factors for small bowel cancer in Crohn's disease

		Protective factor against small bowel cancer?	
		Yes	No
1	Diet	¹ Negri <i>et al</i> ^[25]	² Chow <i>et al</i> ^[77]
2	5-ASA	Piton <i>et al</i> ^[21] Solem <i>et al</i> ^[26]	
3	CD medications		Canavan <i>et al</i> ^[76] Solem <i>et al</i> ^[26] (other than 5-ASA)

¹Coffee, fish, vegetables, fruit; ²Fruits and vegetables. 5-ASA: 5-aminosalicylic acid.

Two important clinical indicators of malignancy are recrudescence symptoms after long periods of relative quiescence and small bowel obstruction that is refractory to medical therapy^[23]. Therefore, it is prudent to consider a surgical assessment of patients with longstanding symptomatic Crohn's disease who fail to respond to conservative management.

The usual age of diagnosis of small bowel carcinoma in Crohn's disease patients is 45 to 55 years^[4,6,7,22,23]. This is in contrast to small bowel carcinoma *de novo* which is usually diagnosed between 60 and 69 years of age^[25]. Crohn's disease will often predate the carcinoma diagnosis by 20 to 25 years^[4,22-24,26].

Palascak-Juif *et al*^[27] studied 20 patients with Crohn's disease-associated small bowel carcinoma recruited from French university hospitals and compared them to 40 patients with small bowel carcinoma *de novo* recruited from a population-based registry. Small bowel carcinoma occurred after a median time of 15 years of Crohn's disease and was located within the inflamed areas of the ileum (19) or jejunum (1), whereas in patients with small bowel carcinoma *de novo* it was distributed all along the small intestine. The median age of diagnosis of small bowel carcinoma was 47 years (range 33-72 years) in patients with Crohn's disease and 68 years (range 41-95 years) in those with small bowel carcinoma *de novo*. The cumulative risk of small bowel carcinoma was 0.2% and 2.2% after 10 and 25 years of small bowel Crohn's disease, respectively. The diagnosis was made preoperatively in 1 of 20 patients with Crohn's disease and 22 of 40 patients with small bowel carcinoma *de novo*. Signet ring cells were found in 35% of Crohn's disease cancers but not in patients with small bowel carcinoma *de novo*. Relative survival at 2 and 5 years was not significantly different between these two categories of patients (54% *vs* 37% and 35% *vs* 30%; with and without Crohn's disease, respectively).

In a retrospective review from 1993 to 2009, Widmar *et al*^[23] identified 29 patients with small bowel carcinoma (22 ileal and 5 jejunal) in Crohn's disease. There were no carcinomas in excluded intestinal loops. The median age of onset of Crohn's disease symptoms was 25 years and the median age at cancer diagnosis was 55.4 years, for a mean interval of 25.3 years. Widmar found that 75% of carcinomas arose in the terminal ileum, a location that

only accounts for 13% of sporadic small bowel adenocarcinomas^[28]. Patients with Crohn's disease developed adenocarcinoma at an average age of 48 years versus 65 in the general population, with a male to female ratio of 3 to 1.

Solem *et al*^[26] described the clinical features, outcomes, and risk factors of small bowel carcinoma in Crohn's disease. Nine cases (4 males) were identified. The patients presented with abdominal pain (89%), obstruction (89%), and weight loss (78%). The carcinoma was located in the ileum in 8 patients (89%) and in the jejunum in 1 patient (11%). All cases but one had advanced disease with either lymph node involvement or metastases. The mortality rates at 1 and 2 years were 42% and 61%, respectively.

Floch *et al*^[29] reviewed 47 previously reported small bowel carcinomas in Crohn's disease. The average age of the Crohn's carcinomas was 46.5 years while that for the *de novo* group was 55 years. The sexual ratios were 2.46:1 and 2:1 males to females for the respective groups. The *de novo* carcinomas had a slight predilection for the duodenum (4.7%) while the latter group had a heavy predilection for the ileum (70.8%) and contained no duodenal carcinomas. The prognosis of the Crohn's group appeared to be much worse than that of the *de novo* group with five year survivals of 3.7 and 20%-22%, respectively. Late diagnosis in the enteritis group was felt to be the major reason for this.

Hoffman *et al*^[7] also reviewed the literature and found 49 cases and added two of their own. The Crohn's associated carcinomas differed from carcinoma not associated with Crohn's in that (1) mean age of carcinoma discovery was less (46 years *vs* 64 years); (2) more carcinomas arose in the ileum (76% *vs* 27%); (3) diagnosis and cure were less successful; and (4) they occurred more frequently.

The review by Fresko *et al*^[30] of 59 reported cases of carcinoma of the small bowel in Crohn's disease, to which they added three of their own cases, revealed that (1) carcinoma develops at a younger age than in carcinoma *de novo*; (2) there is no difference in incidence of carcinoma in the first, second and third decades after onset of symptoms of Crohn's disease; (3) 73% of neoplasms arose in the ileum; (4) in all but one case it developed in inflamed segments of bowel; and (5) in 31% of cases carcinoma developed in a bypassed segment of bowel. They concluded that Crohn's carcinoma is a complication of Crohn's disease and not a chance co-existence of the two diseases in the patient.

DIAGNOSIS

The clinical diagnosis of small bowel carcinoma in Crohn's disease patients based on symptoms and physical examination is quite difficult, if not impossible. Indeed, many patients with carcinoma of the small bowel are not suspected of having a malignancy even at time of operation^[5,6,31-33]. Most cases of small bowel carcinoma have been in segments involved with Crohn's disease. These malignancies were indistinguishable radiologically from

longstanding Crohn's disease. In two of the patients described by Kerber^[4] and Frank^[5], there was "shouldering", destruction, and a mass.

In general, imaging techniques may miss small lesions and may not be able to differentiate areas of small bowel carcinomas from those of severe Crohn's disease. Routine computed tomography (CT) exposes patients to radiation, and although magnetic resonance (MR) imaging does not, it is time consuming and costly^[34]. Buckley *et al*^[35] found CT staging of small bowel carcinoma to be 47% accurate but errors occurred in patients with Crohn's disease. Enteroclysis is invasive and requires special training^[36]. The usefulness of FDG-PET is limited by the background chronic inflammation of Crohn's^[37].

Video capsule endoscopy is challenged by issues of visualization (*i.e.*, limited field of vision, non-continuous image capture, lesions hidden in folds), inadequate preparation, and the stenosing nature of Crohn's disease^[36] that may prohibit a capsule from passing. Furthermore, lesion localization can be difficult and it does not allow for tissue sampling^[38].

Enteroscopic techniques do allow direct visualization and tissue sampling but are invasive, labor intensive, and may be limited by the length and tortuosity of the small bowel. Intraoperative endoscopy is now reserved for lesions which are not accessible by balloon enteroscopy^[39].

Despite their disadvantages, there are published cases demonstrating the utility of some of the techniques listed above. Placé and colleagues^[40] observed two different patterns using MR-enterography: the first was a long, circumferential, asymmetric, and heterogeneous thickening of the ileum with a visible nodule on free induction echo stimulated acquisition images, and the other was a mass of the terminal ileum showing restricted diffusion on diffusion weighted MR imaging. Soyer *et al*^[41] evaluated 7 patients with small bowel carcinoma, and on CT enterography the carcinoma was visible in five patients. Four different patterns were individualized including small bowel mass (2 patients), long stenosis with heterogeneous submucosal layer (2 patients), short and severe stenosis with proximal small bowel dilatation (2 patients), and sacculated small bowel loop with irregular and asymmetric circumferential thickening (1 patient). Stratification, fat stranding, and comb signs were present in 2, 2, and 1 patient(s), respectively. Nevertheless, adenocarcinoma may be completely indistinguishable from benign fibrotic or acute inflammatory strictures.

A case reported by Kodaira *et al*^[42] highlighted the unique successful combination of PET/CT and double balloon enteroscopy for the diagnosis of small bowel carcinoma in a Crohn's disease patient. Van Weyenberg *et al*^[43] found both MR enteroclysis and video capsule endoscopy useful but they believe that MR enteroclysis is the better option. Ultimately, a combination of methods is likely the present day solution.

On the basis of their study of patients in whom adenocarcinoma of the small bowel developed as a complication of Crohn's disease, Kerber^[4] and Frank^[5] concluded: (1) the development of adenocarcinoma is more likely

to be seen in patients with longstanding disease; (2) classical radiographic appearance of carcinoma may not be seen; (3) a progressive change in radiographic appearance over time with the development of masses, fistulas, strictures and obstruction should raise the suspicion of co-existing carcinoma; (4) malignancy should be considered when there is a longstanding quiescent disease activity followed by a recrudescence of symptoms with concurrent radiographic changes; and (5) fistulas may be associated with carcinoma in two ways: a mass produced by carcinoma or carcinoma arising in chronic fistulas from Crohn's disease. Contrary to prior recommendations, they suggest that obtaining radiographic examinations to document changing patterns of disease displays an important role in the management of patients with Crohn's disease and may lead to earlier detection of complicating carcinoma thus improving prognosis in such patients.

PATHOLOGY

In contrast to *de novo* small bowel carcinomas which are most often in the duodenum (55%)^[28], 75% of Crohn's related small bowel carcinomas are ileal^[22,28]. Miller *et al*^[44] noted that all small bowel carcinomas associated with Crohn's disease were ileal in location. Watanabe *et al*^[45] published a summary of small bowel carcinoma within Crohn's disease up until 1991 documenting only adenocarcinomas and signet ring cell carcinomas (Table 3). Petras *et al*^[46] reported four patients with small intestinal carcinoma: three with poorly differentiated or signet ring cell type carcinomas and one with mucinous type. All four patients had high grade dysplasia in the mucosa immediately adjacent to the carcinoma, supporting the dysplasia-carcinoma sequence believed to occur in Crohn's disease as with ulcerative colitis^[47,48]. A wider variety of malignancies have been reported and are noted in Table 3, including sarcomas, lymphomas, and carcinoids.

TREATMENT

The treatment of choice is wide resection of the small bowel segment harboring the carcinoma as well as resection of the corresponding mesentery and lymph nodes^[22]. Pancreaticoduodenectomy for lesions of the second or third portion of the duodenum and right colectomy for carcinoma of the distal ileum would be required^[49].

Evidence regarding the value of adjuvant chemotherapy for small bowel carcinoma is sparse and consists mostly of small retrospective reviews. Most available data is from experience in managing ampullary adenocarcinoma. Fishman *et al*^[50] reported response rates upwards of 30% in the palliative setting: 33% with Gemcitabine, 50% with 5-FU or Capecitabine, and 42% with Platinum- or Irinotecan-based therapy.

PROGNOSIS

The prognosis of Crohn's associated small bowel carcinoma varies among reported studies but has been noted

Table 3 Histopathology of small bowel cancers in Crohn's disease

Histology	Watanabe <i>et al</i> ^[45]	Update since 1991
Adenocarcinoma	61 cases reported up until 1991 as quoted by Watanabe	Barwood <i>et al</i> ^[14] Chan <i>et al</i> ^[78] Chen <i>et al</i> ^[64] Christodoulou <i>et al</i> ^[79] Dossett <i>et al</i> ^[22] Feldstein <i>et al</i> ^[49] Fell <i>et al</i> ^[80] Fielding <i>et al</i> ^[9] Gillen <i>et al</i> ^[81] Gusakova <i>et al</i> ^[82] Jaskowiak <i>et al</i> ^[15] Jess <i>et al</i> ^[73] Kamiya <i>et al</i> ^[69] Katsanos <i>et al</i> ^[83] Kersting <i>et al</i> ^[65] Koga <i>et al</i> ^[84] Kronberger <i>et al</i> ^[85] Lindgren <i>et al</i> ^[86] Mellemjaker <i>et al</i> ^[71] Menon <i>et al</i> ^[12] Michelassi <i>et al</i> ^[55] Palascak-Juif <i>et al</i> ^[27] Partridge <i>et al</i> ^[13] Ribeiro <i>et al</i> ^[11] Richards <i>et al</i> ^[54] Rubio <i>et al</i> ^[87] Sammartino <i>et al</i> ^[88] Sigel <i>et al</i> ^[48] Solem <i>et al</i> ^[26]
Sarcoma		Gollop <i>et al</i> ^[89] (leiomyosarcoma) Jess <i>et al</i> ^[73] (leiomyosarcoma) Fielding <i>et al</i> ^[9] (reticulum-cell sarcoma)
Local lymphoma		Jess <i>et al</i> ^[73]
Carcinoid		Chen <i>et al</i> ^[64] Kvist <i>et al</i> ^[70] Mellemkjaer <i>et al</i> ^[71] Savoca <i>et al</i> ^[62]
Poorly differentiated		Petras <i>et al</i> ^[46] Savoca <i>et al</i> ^[62] Simpson <i>et al</i> ^[47]
Signet ring	8 cases reported up until 1991 as quoted by Watanabe	

to be poorer than the *de novo* small bowel carcinomas^[24]. Most small bowel carcinomas in Crohn's disease present at a younger age and are more diffusely and distally located than *de novo* carcinomas, usually making them undiagnosable at a curable stage. Indeed, two-thirds of cases present with intestinal obstruction. Greenstein^[51] reported two year disease survival for small bowel carcinoma in Crohn's disease as 9% compared with 15%-25% for *de novo* carcinomas. Mortality for carcinoma in excluded bowel has been reported to be as high as 100%^[51]. One report of carcinomas developing in small bowel Crohn's strictures found only 9 such cases^[52]. All patients had Crohn's disease for more than ten years. The average age of patients was 48 years compared to 65 years for *de novo* carcinomas. In patients with Crohn's disease, carcinoma affects the ileum twice as commonly as the jejunum and four times

as commonly as the duodenum. Fifty-nine percent of all carcinomas complicating Crohn's disease were discovered incidentally during pathologic examination of resected specimens. If small bowel alone is considered this figure rises to 70%.

Small bowel carcinomas associated with Crohn's disease tends to be poorly differentiated and are associated with a poor prognosis^[4,5,19,33,53]. Two year survival rates have been found to be as low as 27%^[22]. In a report by Richards^[54] with three ileal carcinomas, survival ranged from 8 to 44 mo.

Michelassi *et al*^[55] reported 14 cases of intestinal carcinoma complicating Crohn's disease, 7 occurring in the small intestine and 7 in the large bowel. Two thirds of patients were male. The average age at time of diagnosis of Crohn's disease and carcinoma was 28 and 48 years respectively. In five patients with small bowel carcinoma the diagnosis was made at laparotomy. In the remaining cases only careful histologic examination revealed the carcinoma. Six small bowel carcinomas were located in the ileum. Two small bowel carcinomas were multi-focal and had surrounding mucosal dysplasia. No patient with regional or distal metastases survived five years in comparison with an 83% five year actuarial survival rate in patients with carcinoma confined to the intestinal wall. Mean survival was 6 mo for patients with small bowel carcinoma.

Hawker *et al*^[53] reported the clinical and pathological details of three cases diagnosed between 1968-1980 with a review of 58 patients from the literature. Of the 61 cases, 41 carcinomas occurred in the ileum, 18 in the jejunum, 1 in the duodenum and ileum, and 1 in the ileum and colon. Eighteen occurred in bypassed intestinal loops. The prognosis was poor: 44 patients (72%) died with a mean interval of only 7.9 mo from the diagnosis of their malignancy.

Widmar *et al*^[23] reported significant differences in the two year survival for node negative versus node positive carcinomas (79.3% *vs* 49%) and for localized versus metastatic disease (92.3% *vs* 33.3%). Overall, 36 mo survival was 69.3% compared to 40% among those without excluded loops. Sixteen patients had long periods of quiescent disease before the diagnosis (7-45 years) and 16 required operation for bowel obstruction that was refractory to medical management.

OUR EXPERIENCE

From 1990 to 2013, 10 patients with underlying Crohn's disease and small bowel adenocarcinoma were treated at our institution. In our series, there were twice as many males as females. The median age of Crohn's diagnosis was 28 years and the median age of small bowel adenocarcinoma diagnosis was 57 years; this interval is consistent with the literature. In none of the 10 patients was the diagnosis known pre-operatively. Nine patients presented with a clinical picture of obstruction that did not respond to steroid treatment and required operation. Of the nine, two were found to have metastatic disease secondary to small bowel adenocarcinoma

at the time of operation, while the diagnosis for the remaining seven was made on final pathology examination. The tenth patient in our series had refractory Crohn's disease and was incidentally found to have terminal ileal adenocarcinoma on final pathology.

An equal number of patients had Crohn's disease isolated to their small bowel as concomitant small and large bowel disease. None of our patients had bypassed loops of small bowel. In fact, only three patients had had previous operations for Crohn's disease. All but one of our patients had terminal ileal adenocarcinomas; the exception was one jejunal carcinoma. Moreover, all patients had either stricturing and/or fistulizing Crohn's disease. Four of our 10 patients had been treated with 5-ASA, a reported protective factor. All patients had a history of remote immunomodulator use but none were on maintenance immunomodulators at the time of presentation; if they were receiving medical therapy, it was solely high dose steroids. This is consistent with the conclusions made by Kerber^[4] and Frank^[5] concerning the development of a small bowel adenocarcinoma after a period of quiescent Crohn's disease. As reported by others, the prognosis for small bowel adenocarcinoma in our series was also quite poor: the carcinoma-related mortality in our series is 70%.

SURVEILLANCE

It has become increasingly recognized that the risk of developing carcinoma of the colon in patients with colonic Crohn's disease is comparable to those with chronic ulcerative colitis. Hence, regular colonoscopic surveillance in search of dysplastic changes is in order. However, no similar surveillance for patients with small bowel Crohn's disease is possible.

Greenstein^[24] suggests that surveillance should consist of regular abdominal examinations and that the recurrence of obstructive symptoms as well as the development of new symptoms should not be ignored especially after long quiescent periods.

CEA levels, found to be elevated in up to 38% of patients with active Crohn's disease^[56,57], have not been found to be useful in monitoring for small bowel carcinoma^[19,58].

LIMITATIONS

Each of the studies included in this descriptive view has its own limitations. A descriptive review such as ours could not possibly be exhaustive if it had strict inclusion/exclusion criteria. Thus we chose to include data and observations from all available studies despite their limitations.

It is difficult to draw conclusions regarding the cumulative incidence of small bowel carcinoma in Crohn's disease from studies with such a low frequency of event as small bowel carcinoma. Compared to current data, incidence values from older studies may actually be overestimated, as 5-ASA formulations (possibly protective against small bowel carcinoma) were released in the

late 70's and 80's, late in the observation period of most cohorts^[59]. Furthermore, the majority of reports did not have small bowel carcinoma as a primary outcome. Analysis did not routinely address incidence and discussions were sometimes not exclusive to the small bowel (e.g., "intestinal"^[60], "upper digestive tract"^[61], or discussing risk factors for small bowel and colorectal carcinomas together^[62]). Studies rarely controlled for immunosuppressive agents, tobacco or alcohol^[63], and those that did examine such exposures did not provide quantitative data^[64]. Finally, multiple biases inherent of retrospective and single-centre studies exist in the available literature. For example, a high incidence of small bowel carcinoma in Crohn's disease may reflect a bias in tertiary hospitals^[65] or a surveillance bias due to close monitoring of Crohn's disease patients^[66], and reported risk factors may be a result of recall bias^[67]. These limitations are inherent to the challenging problem of a rare disease that is difficult to diagnose.

CONCLUSION

In this review, we highlighted the available current evidence and the gaps of knowledge, technology, and clinical guidelines required for improving care of Crohn's disease patients at risk of this devastating problem. Although the association of carcinoma in Crohn's disease and the need to screen Crohn's disease of the colon is well established, carcinoma associated with Crohn's disease of the small bowel is difficult to diagnose and indeed is often not identified until operation for what is believed to be an exacerbation or non-response to medical therapy. Sadly, the diagnosis is often made after careful examination of the resection specimen by the pathologist. Over the decades there has been a lack of significant improvement in prognosis. There is a need to elucidate screening modalities to facilitate earlier diagnosis and treatment.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease**Ulcerative colitis: From inflammation to cancer. Do estrogen receptors have a role?**

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Abstract

Ulcerative colitis (UC) is a condition at increased risk for colorectal carcinoma (CRC) development. Nowadays, screening and follow-up programs are routinely performed worldwide to promote the early detection of CRCs in subjects with well known risk factors (extent, duration and severity of the disorder). The diffusion of these procedures is presumably the main reason for the marked reduction of cancer incidence and mortality in the course of UC. In addition, chemoprevention has been widely investigated and developed in many medical fields, and aspirin has shown a preventive effect against CRC, while mesalazine has been strongly invoked as a potential chemopreventive agent in UC. However, available studies show some limitations due to the obvious ethical implications of drug withdrawal in UC in order to design a control group. The estrogen

receptors (ER) alpha/beta balance seems to have a relevant influence on colorectal carcinogenesis and ER beta appears to parallel apoptosis, and hence an anti-carcinogenic effect. Phytoestrogens are compounds acting as ER beta agonists and have shown a promising chemopreventive effect on sporadic as well as genetically inherited CRC. There is evidence suggesting a role for ERs in UC-related carcinogenesis. In this perspective, since these substances can be considered as dietary supplements and are completely free from side effects, phytoestrogens could be an interesting option for CRC prevention, even when the disease is a consequence of long-term chronic inflammation, as in the course of UC. Further studies of their effects are warranted in both the basic research and clinical fields.

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Key words: Ulcerative colitis; Epithelial dysplasia; Colorectal cancer; Estrogen receptors; Chemoprevention; Phytoestrogens; Dietary supplementation; Inflammatory bowel disease

Core tip: The present work outlines the main data regarding a possible involvement of estrogen receptors in colorectal carcinogenesis, paying particular attention to cancer arising in the course of ulcerative colitis. A protective role for beta receptors has been suggested by many studies. The challenge for the future could be to devise chemopreventive strategies against colorectal carcinoma employing estrogen receptor beta agonists, such as phytoestrogens.

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ULCERATIVE COLITIS: FROM INFLAMMATION TO CANCER

Ulcerative colitis (UC) is associated with an increased risk of colorectal cancer (CRC), which has been related to the long-standing chronic inflammation^[1]. However, the magnitude of the risk is difficult to estimate, as many factors may bias study results^[2] (*i.e.*, patient selection, number of patients, completeness of case recruitment and ascertainment and duration of follow-up)^[2,3].

Castaño-Milla *et al.*^[4] reported an overall incidence rate of CRC in UC of 1.67/1000 per year of disease (PYD) and incidence rates per decade were estimated at 1.01/1000, 3.75/1000 and 5.85/1000 PYD for the first, second and third decades, respectively. In a meta-analysis of prospective population-based studies, Jess *et al.*^[5] found that an average of 1.6% of patients with UC were diagnosed with CRC during the first 14 years of follow-up, and the estimated standardized incidence ratio (SIR) was 2.39 (2.1-2.7). Recent time-trend studies also demonstrate a decreasing risk of CRC in UC patients^[6]. In a recent meta-analysis^[4] the incidence rate was found to have decreased from 4.29/1000 PYD in studies published in the 1950s to 1.09/1000 PYD in the studies published between 2000 and 2011.

As known, reported risk factors for CRC include extensive disease^[7,8], young age at diagnosis^[9], a family history of CRC^[10], co-existing primary sclerosing cholangitis (PSC)^[11] and persistent inflammation of the colon^[12,13].

The pathophysiology of colitis-associated cancer suggests the action of numerous positive and negative regulators^[14]. Positive regulators are pro-carcinogenic cytokines such as tumor necrosis factor alpha (TNF alpha), that is over-expressed in a murine model of carcinoma arising on colitis^[15], interleukin (IL)-6^[16] and IL-21^[17] and chemokines such as CCL2, whose expression is enhanced by TNF alpha, causing the recruitment of macrophages and monocytes^[18]. Negative regulators include IL-10^[19,20], transforming growth factor beta (TGF beta)^[21] and MyD88, a Toll-like receptor adaptor, that has been found to significantly reduce tumor number and size in the Apc^{min/+} mouse model of intestinal tumorigenesis^[22,23].

The progression from UC to CRC is a multistep process in which the accumulation of genetic mutations leads to the sequential evolution to low-grade dysplasia (LGD), high-grade dysplasia (HGD) and finally to cancer^[24]. The p53 tumor suppressor gene appears to be a key factor in the initial steps of UC-associated colorectal carcinogenesis, being the most frequent single founding mutation in UC associated CRC^[25]. p53 is overexpressed in 33%-67% of patients with dysplasia and in 83%-95% of patients with UC-associated CRC^[26,27]. Other genes that undergo mutation in the following stages of carcinogenesis are kRAS, DCC, cyclin D, COX, iNOS, APC

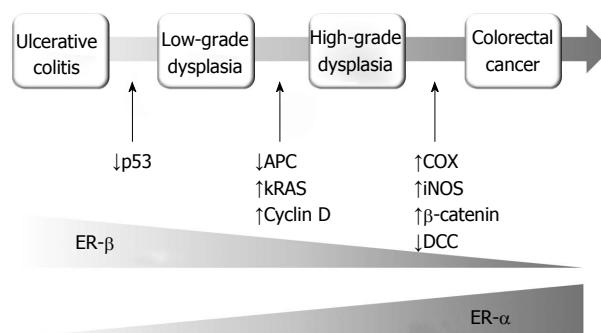


Figure 1 progressive steps of ulcerative colitis-related carcinogenesis, genetic pathways and estrogen receptors alpha and beta patterns.

and beta-catenin (Figure 1), in a sequence that is substantially different from the classical adenoma-carcinoma pathway^[28,29].

The essential morphological features of dysplasia, are (1) nuclear alterations such as increased nuclear to cytoplasmic ratios and hyperchromasia; (2) depletion of goblet cells; and (3) abnormal architectural patterns corresponding to dysregulated cellular proliferation, such as glandular crowding, a villous architecture and diminished surface maturation. HGD differs from LGD in that there are additional alterations, *i.e.*, impaired cellular polarity including loss of nuclear parallelism, stratification of nuclei patterns such as a cribriform architecture. In most cases, the nuclei in HGD show severe cytological aberrations such as irregular nuclear membranes, abnormally prominent nucleoli or atypical mitotic figures^[30]. The progression of such alterations is accompanied by both a progressive increase of epithelial proliferation and a reduction of apoptosis. This phenomenon starts as alterations of glandular architecture (*i.e.*, shortening, loss of parallelism, ramification and branching) which anticipate the dysplasia onset^[31].

The potential risk of malignant degeneration of UC to CRC has made it necessary to institute surveillance protocols to achieve early recognition and treatment of dysplastic lesions. The current evidence-based consensus for endoscopy in inflammatory bowel disease^[32] suggests that surveillance should start when the risk starts to increase, *i.e.*, after 8-10 years from the onset of disease^[7]. This first colonoscopy also aims to reassess the extent of disease, since this parameter has an impact on the risk of CRC. After this first colonoscopy, patients with high risk features (stricture or dysplasia detected within the past 5 years, PSC, extensive colitis with severe active inflammation, or a family history of CRC in a first degree relative aged less than 50 years) should undergo surveillance colonoscopy annually. Conversely, patients with intermediate risk factors should have surveillance colonoscopy scheduled every 2 to 3 years and those without risk factors every 5 years. Biopsy sampling is fundamental: the American Gastroenterological Association recommends extensive sampling, of a minimum of 33 specimens^[33], while, according to the British Society of Gastroenterology^[34], two to four random biopsies every

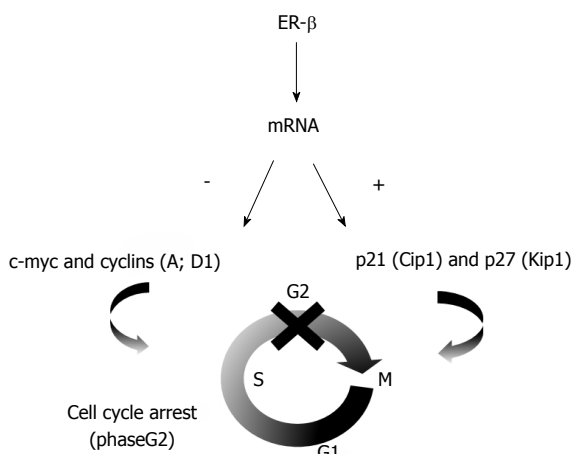


Figure 2 Estrogen receptors beta and interactions with genes involved in the regulation of cell cycle. Estrogen receptors (ER) beta has an antagonist inhibitory function, mediated by the down-regulation of proto-oncogenes c-myc and cyclins (as indicated by minus sign next to the arrow on the left side) and up-regulation of oncosuppressants p21 and p27 (as indicated by plus sign next to the arrow on the right side). In the lowest part of the figure is indicated the cell cycle phases and the site of its interaction with ER beta induced mediators (G2 phase).

10 centimetres should be taken.

Compliance to surveillance protocols, as well as a correct clinical overview of UC and the adequate pharmacological management of the disease, have led to a decreasing CRC incidence and mortality in UC^[35,36]. In 1971, de Dombal^[37] reported a 5% cumulative risk of CRC in a population from Leeds with extensive UC after 10 years and 41.8% after 25 years. Thirty years later, the cumulative risks reported by Lakatos *et al.*^[38] had dropped dramatically: 0.6% after 10 years, 5.4% after 20 years and 7.5% after 30 years of disease duration. These data testify to the exceptional impact of surveillance in the natural history of UC^[39], but we must consider that it is not the only prevention strategy: other routes, such as chemoprevention, may have a remarkable effect.

ESTROGEN RECEPTORS

Modern medicine and oncology have been profoundly affected by the discovery of the estrogen receptors (ERs), a potential marker that plays a pivotal role in the pathogenesis, prognosis and therapy of various cancers, such as breast, prostate and colon. Estrogens can regulate the growth, differentiation, and function of various target tissues both within and outside the reproductive system^[40,41]. The most relevant event after the initial discovery of these receptors^[42] was the identification of two subtypes, ER alpha and ER beta, that are expressed at different levels in each organ of the human body^[43]. Variations in the phenotype of knock-out mice lacking ER alpha or ER beta suggested that these receptors have different biological activities^[44]. Moreover, *in vitro* and *in vivo* studies in ER beta knock-out mice demonstrated that ER beta is a modulator of ER activity, as it is able to reverse the effects of ER alpha and to inhibit estradiol-dependent

proliferation^[45,46]. These experiments demonstrated that ER alpha is a positive regulator of cellular growth, while ER beta has an antagonist inhibitory function, mediated by the down-regulation of proto-oncogenes (c-myc and cyclins) and up-regulation of oncosuppressants (p21 and p27), resulting in cell cycle arrest^[47] (Figure 2). Experiments showing that in various cancers ER alpha is over-expressed and ER beta is down-regulated confirmed *in vitro* studies and demonstrated that cell proliferation is the result of a balance of ER alpha and ER beta^[48,49].

ESTROGEN RECEPTORS AND COLORECTAL CANCER

The hypothesis of a possible link between CRC and ERs was advanced after the publication of epidemiological studies showing that females have a lower rate of colonic adenomas and cancers than males before menopause and that the differences progressively lessen after menopause^[50]. Similarly, both observational and interventional data have shown that hormone replacement therapy decreases colonic adenoma and cancer risks^[51,52]: in the last 40 years, a reduction of deaths from large bowel carcinoma has been observed in the United States. This reduction was significantly higher in women (30%) as compared to men (7%). In the same study, a link was observed between oral contraceptive use and a reduction of colorectal cancer, whereas there was a higher than expected frequency of colorectal tumors among non users^[53].

After the demonstration by our group that ERs are expressed in the colonic mucosa^[54], Konstantinopoulos *et al.*^[55] demonstrated that ER beta is highly expressed in normal colonic mucosa in humans, while it is significantly reduced in CRC; this reduction is more pronounced in the case of poorly differentiated tumors. Since the majority of CRCs are derived from adenomatous polyps (a precancerous condition) our group recently evaluated the expression of ER alpha and ER beta in the colonic tissue of 25 patients with adenomatous polyps of the colon and in 25 normal subjects^[56]. ERs expression was then correlated to proliferation and apoptosis. Our data confirmed that ER beta is the prevalent estrogen receptor in normal mucosa and shows a significantly reduced expression in adenomatous polyps (Figure 3). In a successive study, we confirmed that ER beta plays a primary role in the regulation of colonic mucosa proliferation in patients affected by Familial Adenomatous Polyposis (FAP)^[57], an inherited disease characterized by an early inclination to develop hundreds of polyps and consequently CRC. Furthermore, ERs can even influence the prognosis of CRC, as it has been demonstrated that patients affected by CRCs with a minimal ERs expression had poor prognosis and short survival^[58].

All these data confirm that sex steroid hormones are involved in CRC development and suggest that ER beta could play an important role in the early phase of the carcinogenic process and hence could be a target in the primary prevention of CRC^[59].

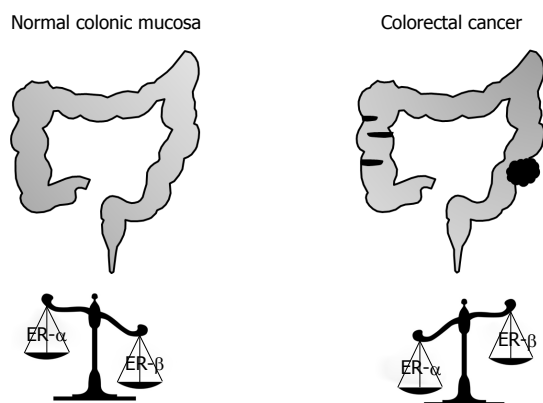


Figure 3 Alpha and beta estrogen receptors balance in normal and neoplastic colon.

ESTROGEN RECEPTORS EXPRESSION IN THE PROGRESSIVE STAGES OF ULCERATIVE COLITIS-RELATED CARCINOGENESIS

ER beta has been suggested to exert anti-inflammatory and anti-tumorigenic effects in the colon, providing a translational potential to prevent and/or treat inflammatory bowel disease (IBD) and its progression to colitis-associated CRC^[60,61]. Most studies in this field used a consolidated animal model which accurately mimics the carcinogenic model related to chronic bowel inflammation in mice (*i.e.*, Azoxymethane/Dextran Sodium Sulfate - AOM/DSS)^[62,63].

Saleiro *et al.*^[64] demonstrated that ER beta-deficient mice developed more severe clinical colitis compared to wild type mice, as evidenced by a significantly higher disease activity index after DSS treatment, as well as the inflammation score and grade of dysplasia. ER beta-deficient colons presented a greater number and size of polyps, and were characterized by a significant increase in IL-6, IL-17, TNF alpha and interferon-gamma mRNA levels as compared to wild type mice organs. Furthermore, higher protein expression levels of nuclear factor-kappa B, inducible nitric oxide synthase (iNOS), beta catenin, proliferating cell nuclear antigen, mucin-1, and significantly lower caveolin-1 and mucin-2 protein levels, were shown in ER beta knock-out mice compared to wild type. These data suggest a possible anti-inflammatory and anti-neoplastic mechanism of action of ER beta in UC-arisen CRC. These results suggest that ER beta may be protective in the AOM/DSS-induced CRC model in mice, supporting a preventive and/or therapeutic potential for the use of ER beta-selective agonists in IBD.

Fujii *et al.*^[65] performed a study to clarify whether methylation analysis of the ER gene in non-neoplastic epithelium can contribute to the prediction of an increased neoplasia risk in UC patients. The study was based on the assumption that the ER gene shows an age-related methylation in the colorectal epithelium and this phenomenon is frequently found in sporadic colorectal

neoplasia, suggesting that it may predispose to colorectal neoplasia. The results suggested that the analysis of ER gene hypermethylation may be a potentially useful marker for identifying individuals at increased risk of neoplasia among those with long-standing and extensive UC. The same group confirmed that the quantitative analysis of ER gene methylation in non-neoplastic epithelium is a marker for identifying individuals at increased risk of neoplasia in long-standing and extensive UC^[66].

A preliminary report by our group^[67] assessed the pattern of ER-alpha/beta expression in relation to epithelial apoptosis and cell proliferation in long-lasting UC. We did not observe significant variations in ERs and their ratio in UC compared to UC-low degree dysplasia. However, there was a statistically significant progressive increase in apoptosis in UC and in UC-dysplasia that, despite Ki-67 expression, revealed a more marked significant increase at the same stages. This result, despite the small sample and the inclusion of only low-grade dysplasia, suggested that a possible ER-beta overseer of apoptosis/proliferation is operative until the investigated stage of carcinogenesis (Figure 1). In fact, in LGD we observed a high increase in cell proliferation with invariable levels of ER beta, accompanied by mild increased apoptosis, that was presumably unable to completely counter Ki-67 over-expression. Further, we investigated ER beta, ER alpha expression and their ratio in normal mucosa, in UC and in UC-low and high grade dysplasia and CRC. ERs did not show significant changes until LGD, while in HGD and UC-carcinoma there was a dramatic loss of ER beta expression and the ER beta/ER alpha ratio. Apoptosis and the TUNEL/Ki-67 ratio demonstrated a statistically significant progressive decrease from LGD to UC-carcinoma^[68].

IS THERE A ROLE FOR CHEMOPREVENTION?

The main risk factors for colorectal cancer are not suitable targets for therapeutic intervention, but primary chemoprevention is an intriguing therapeutic option. The question whether mesalazine could exert a chemopreventive effect has been raised and various studies have investigated this aspect.

The mechanisms by which aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) act in the chemoprevention of CRC in non-IBD patients have not been entirely elucidated. However, data on the chemopreventive effect of aspirin and NSAIDs and CRC are supported by a series of independent lines of evidence. Indeed, several epidemiological studies have shown an inverse correlation between aspirin intake and the risk of CRC^[69-71]. Furthermore, studies on secondary chemoprevention reported that aspirin intake was associated with a decreased risk of adenoma recurrence^[72,73]. Aspirin and NSAIDs seem to act by inducing apoptosis in the colonic epithelium through the inhibition of cyclooxygenase (COX) activity and arachidonic acid accumulation^[74]. Recent evidence suggests that COX inhibition can also change the activity

of mitogen-activated protein kinases and NF κ B^[75,76].

The analogies between acetyl-salicylic acid and mesalazine (5-amino-salicylic acid), and the results obtained by using acetyl-salicylic acid as a chemopreventive agent in patients with sporadic colorectal cancer have prompted the study of potential chemopreventive effects of mesalazine in inflammatory bowel disease. The results of both epidemiological and experimental studies have shown that long-term 5-amino-salicylic acid treatments appear to have a chemopreventive effect. We can cite two studies, by Eaden and Lashner, in which the relative risk of CRC was estimated to be 0.18 and 0.88, respectively^[77,78]. In a group of patients affected by UC and PSC, the risk was 0.88^[79]. The evidence for this effect is provided by retrospective and case-control studies, however, whose results do not reach the highest grades for evidence-based recommendations. Indeed, not all clinical studies reported favorable results regarding CRC in IBD patients. Negative results were mainly reported in studies that elicited positive results with other drugs such as folate or ursodiol^[80]. The peculiarities of the cohorts enrolled in these studies (disease refractory to conventional therapy, consideration for treatment with experimental therapy, consultation for surgery) may account for the negative outcome.

Positive results are supported by a series of experimental studies demonstrating the multiplicity of actions of 5-amino-salicylic acid, although data regarding the chemopreventive effect of 5-amino-salicylic acid may not be rigorous enough to meet the criteria for the highest evidence-based medicine recommendations. A final consideration is that suitable evidence may not be rationally gained in this case, because discontinuation of 5-amino-salicylic acid treatment would be unethical in patients with UC^[81].

FUTURE PERSPECTIVES OF CHEMOPREVENTION BY BETA RECEPTOR AGONISTS

The data summarized in the previous sections suggest the hypothesis that the loss of ER beta expression could be a marker of colonic mucosa at increased risk for colonic neoplasia and that the induction of ER beta with ER beta-selective phytoestrogens could exert a chemopreventive effect against CRC.

Observational data also suggest that phytoestrogen intake may be associated with a decreased incidence of advanced lesions in both men and women^[82-84]. The mechanism of the putative protective effect of estrogens and phytoestrogens on colonic neoplasia is not fully understood, but it seems to be markedly different from the one underlying the detrimental effect of estrogens in breast cancer. In the breast, it is well established that the detrimental effect is due to estrogen binding to the proliferative ER alpha, since a similar effect is not found in women with ER-negative breast cancers^[85].

Barone *et al.*^[86] have shown that the ER beta/ER alpha ratio was lower in the normal small intestinal mucosa of APC^{min/+} mice than in syngenic APC wild type and this phenomenon was associated with a decreased apoptotic activity. The ER beta/ER alpha ratio and apoptosis were normalized by supplementation with a combination of silymarin and insoluble fibers. The combination also markedly decreased the number and size of intestinal tumors in APC^{min/+} mice^[86]. Silymarin displays a full ER beta agonist activity^[87,88] and lignans also exert phytoestrogenic activity^[89]. Another study by our group^[90] was a randomized, double blind placebo-controlled trial in patients undergoing surveillance colonoscopy for previous sporadic colonic adenomas. Sixty eligible patients were randomized to receive a placebo or active dietary intervention with phytoestrogen supplements twice a day, for sixty days before surveillance colonoscopy. The phytoestrogen administration group showed a significant increase in ER beta protein and a general trend to an increase in ER beta, ER beta/ER alpha, TUNEL/Ki-67 ratio. Moreover, a significant increase of ER-beta protein, mRNA and labeling index (*i.e.*, the percentage of ER-beta positive cells at immunohistochemistry) and a decrease of ER-alpha protein, as well as an increase in ER beta/ER beta protein were observed in phytoestrogen versus placebo group in patients without recurrent polyps. Therefore, the role of ER beta on the control of apoptosis, as well as its amenability to dietary intervention, were supported by this study.

Finally, 90-d supplementation with phytoestrogens was efficacious in reducing polyp number and size in recurrent duodenal adenomas of patients with FAP with an ileal pouch-anal anastomosis^[91].

CONCLUSION

UC is a condition that increases affected patients' risk for CRC development. Nowadays, specific screening and follow-up programs, based on epidemiological and clinical parameters, are routinely performed to promote the early detection of CRC onset. This practice has induced a marked reduction of the cancer incidence and mortality in subjects with UC.

Chemoprevention is an interesting topic which has been widely investigated and developed in many medical fields^[92]. Aspirin has shown a preventive effect on CRC onset, and mesalazine has been strongly invoked as a potential chemopreventive agent against carcinoma arising in UC^[93].

The ER alpha/beta balance seems to have a relevant influence on colorectal carcinogenesis and ER beta appears to parallel apoptosis, thus exerting an anti-carcinogenic effect^[94]. In preliminary studies phytoestrogens, which are able to act as ER beta agonists, have shown promising chemopreventive effects on sporadic as well as genetically inherited CRC. In view of the strong evidence of a role for ERs in UC-related carcinogenesis, and taking into account the fact that phytoestrogens can

be considered as dietary supplements and are completely free from side effects, they offer interesting prospects for CRC prevention even when the disease is the long term consequence of chronic inflammation.

In conclusion, ERs have a role in the development of all different types of CRC^[95] (sporadic, genetic and post-inflammatory). Their targeted use is, therefore, a fascinating field for both basic and clinical investigations in order to elucidate the underlying pathophysiological, prognostic and therapeutic aspects.

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Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease

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Abstract

It has been presumed that aberrant immune response to intestinal microorganisms in genetically predisposed individuals may play a major role in the pathogenesis of the inflammatory bowel disease, and there is a good deal of evidence supporting this hypothesis. Commensal enteric bacteria probably play a central role in pathogenesis, providing continuous antigenic stimulation that causes chronic intestinal injury. A strong biologic rationale supports the use of probiotics and prebiotics for inflammatory bowel disease therapy. Many probiotic strains exhibit anti-inflammatory properties through their effects on different immune cells, pro-inflammatory cytokine secretion depression, and the induction of anti-inflammatory cytokines. There is very strong evidence supporting the use of multispecies probiotic VSL#3 for the prevention or recurrence of post-operative pouchitis in patients. For treatment of active ulcerative colitis, as well as for maintenance therapy, the clinical evidence of efficacy is strongest for VSL#3 and *Escherichia coli* Nissle 1917. Moreover, some prebiotics, such as germinated barley foodstuff, *Psyllium* or oligofructose-enriched inulin, might provide some benefit in patients with active ulcerative colitis or ulcerative

colitis in remission. The results of clinical trials in the treatment of active Crohn's disease or the maintenance of its remission with probiotics and prebiotics are disappointing and do not support their use in this disease. The only exception is weak evidence of advantageous use of *Saccharomyces boulardii* concomitantly with medical therapy in maintenance treatment.

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Key words: Gut; Microbiota; Inflammatory bowel disease; Probiotic; Prebiotic

Core tip: Intestinal microbiota seems to play an important role in the pathogenesis of inflammatory bowel disease. There is very strong evidence supporting the use of certain probiotics and prebiotics in the therapy of ulcerative colitis and pouchitis, whereas their beneficial role in Crohn's disease has not yet been proven. This article describes the role of gut microbiota in the pathogenesis of inflammatory bowel disease and delineates the possible mechanisms of certain probiotics and prebiotics in disease treatment and maintenance of remission.

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REVIEW OF THE FACTS ON GUT MICROBIOTA IN INFLAMMATORY BOWEL DISEASE

A variety of factors, which may be environmental, genetic, immunological, and microbial in nature, contribute to

the development of inflammatory bowel disease (IBD)^[1]. Although the exact etiology of IBD remains unclear, it is believed to be the result of complex aberrant immune responses to as yet undetermined environmental factors (most likely intestinal microorganisms) in the gastrointestinal tract of genetically susceptible hosts^[2].

The human gut normally hosts roughly 10^{14} bacterial organisms of up to 1000 different species; this bacterial community can add up to 1–2 kg^[1]. In total, the number of intestinal bacteria is approximately ten times the number of cells constituting the human body, with the collective bacterial genome, also referred to as the microbiome, containing 100-fold more genes than the entire human genome^[3,4]. More than 99% of the gut microbiota is composed of species within 4 bacterial divisions: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*^[5,6]. Greater variations exist below the phylum level, and certain butyrate-producing bacteria, including *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Bacteroides uniformis*, have been identified as key members of adult gut microbiota^[7]. The predominant species in the proximal small intestine are aerobic and Gram-positive. In the distal small bowel, Gram-negative species begin to outnumber Gram-positive bacteria^[8]. Distally to the ileocecal valve, bacterial concentrations increase sharply^[8], and the most densely populated region of the gastrointestinal tract is the colon, with up to 10^{12} bacteria per gram of intestinal content and a population consisting predominantly of the *Bacteroides*, *Bifidobacteria*, *Fusobacteria*, *Clostridia*, and *Peptostreptococci* groups^[1]. The majority of intestinal bacteria belong to the phyla *Bacteroidetes* (64% of attached colonic species) or *Firmicutes* (23% of normal species)^[1,5]. *Enterobacteriaceae* such as *Escherichia coli* are relatively minor components of the *Proteobacteria* division (8% of all bacteria)^[5].

There is plenty of evidence supporting the hypothesis of the involvement of intestinal microbiota in IBD pathogenesis. Crohn's disease (CD) and ulcerative colitis (UC) tend to occur in the colon and distal ileum, which contain the highest intestinal bacterial concentrations^[5]. A pathogenic role of luminal constituents is suggested by the prevention and treatment of Crohn's disease by the diversion of fecal stream and reactivation of inflammation within one week following reinfusion of ileostomy contents^[9]. In patients with CD, division of the fecal stream proximally to the inflamed mucosa results in reduction of inflammation and induction of healing in the excluded parts of the gut, while relapse occurs with restoration of fecal stream and re-exposure to luminal contents^[9,10]. Similarly, ulcerative colitis patients who undergo ileal pouch-anastomosis surgery develop mucosal inflammation after bacterial colonization of the pouch^[11]. A recent meta-analysis by Khan *et al.*^[12] has shown the significant beneficial effects of antibiotics over placebo for induction of remission in both CD and UC. Antibiotic treatment also appears to provide clinical benefits in patients with CD and inflammation of the ileal pouch^[13]. Furthermore, there are many studies on animal models supporting the role of gut microbiota in the development

of IBD. In experimental animal models of IBD, genetically-engineered animals developed spontaneous colitis under standard laboratory conditions, but remained colitis-free when they were raised in a sterile, germ-free environment, thus indicating that bacterial exposure and colonization are essential for the development of colitis^[13–16]. Additionally, it has been shown that animal models with chemically induced colitis do not develop intestinal inflammation if they are pretreated with antibiotics^[17].

The majority of genes found to be associated with an increased risk for the development of IBD are those encoding proteins that act to preserve the mucosal barrier and/or regulate the host immune system. A major breakthrough in understanding the linkage between genetic predisposition and IBD development was the discovery of the NOD2/CARD15 gene, which encodes a protein belonging to the family of pattern-recognition receptors responsible for microbial recognition, induction of antimicrobial genes, and control of the host adaptive immune response^[18]. The genetic defects found in IBD CD patients might make these individuals particularly susceptible to infection by intracellular bacteria such as *Mycobacterium avium paratuberculosis*, *Listeria monocytogenes*, and adherent-invasive *Escherichia coli*^[19]. Mutations in genes for toll-like receptors, as well as for the CARD4/NOD1 receptor, may also be associated with increased susceptibility for IBD^[20–23].

Patients with CD have increased intestinal permeability, which could reflect mucosal barrier defects that promote bacterial translocation through the intestinal mucosa^[24]. The intestinal mucus barrier is significantly altered in UC patients, particularly in terms of mucus composition and phospholipid concentration^[25]. Altered function of defensins, antimicrobial peptides with bactericidal activities, might also be involved in IBD^[1,24].

Despite much evidence that intestinal microorganisms are required for the triggering and perpetuation of inflammation in IBD, it still remains enigmatic whether a single specific microorganism or a group of microbial agents sharing distinctive characteristics could be responsible, or if it is actually the aberrant immune response to the dysbiosis of the commensal intestinal microbiota that plays the most major role.

Mycobacterium avium paratuberculosis used to be a particularly strong candidate as the single etiologic agent in CD in the past, since it has been shown to cause granulomatous enterocolitis in cattle that closely resembles CD in humans^[26]. However, a two-year trial of combined antibiotic therapy with clarithromycin, rifabutin, and clofazimine (drugs efficient against *Mycobacteria*) did not reveal any difference in disease activity in CD patients with or without antibiotic treatment^[27]. Increased numbers of invasive mucosa-associated or even intramucosal *Escherichia coli* (*E. coli*) have been reported in patients with CD and UC; a new potentially pathogenic group called adherent-invasive *E. coli* (AIEC)^[20,22,28–30]. AIEC are able to adhere to and invade intestinal epithelial cells with a macropinocytosis-like process. They are capable of surviving and

replicating within macrophages, and are known to induce the release of large amounts of pro-inflammatory cytokines, such as TNF- α , by the infected host cell^[23].

Although microbial pathogens have been postulated to cause Crohn's disease and ulcerative colitis since their original descriptions, it is now generally accepted that commensal enteric bacteria, either incidentally or specifically, play an important or even central role in the pathogenesis of inflammatory bowel disease, and provide the constant antigenic stimulation that continuously activates pathogenic T cells to cause chronic intestinal injury^[1,5]. Four broad mechanisms have been proposed to drive pathogenic immunologic responses to luminal microbial antigens: microbial pathogens inducing intestinal inflammation, dysbiosis of commensal microbiota with a decreased ratio of protective/aggressive commensal bacterial species, host genetic defects in containing commensal microbiota, and defective host immunoregulation. These mechanisms increase exposure of bacterial antigens to mucosal T cells or alter host immune responses to commensal bacteria^[5].

In normal hosts, commensal bacteria activate a sequential program of homeostatic responses by epithelial cells, macrophages, dendritic cells, and T and B lymphocytes that permit coexistence with microbes and their products^[5,31,32]. In IBD, genetically predisposed individuals appear to lose the normal tolerance to commensal bacteria, leading to a chronically active inflammation process in which the microbiota provide constant stimulus for the host immune system, causing perpetuation of the disease^[17]. Tissue damage might result from an immunologic misperception of indigenous flora as dangerous organisms or from the failure of normal regulatory constraints on mucosal immune responsiveness to intestinal bacteria^[33]. There is growing evidence that the interplay between intestinal microbes and the mucosa of susceptible individuals triggers a cascade of reactions that starts with the interaction of microbes with specific receptors on intestinal epithelial cells, dendritic cells, and other antigen-presenting cells, followed by the interaction of these activated cells with lymphocytes, resulting in their differentiation into different subsets, driving either Th1 or Th2 inflammatory responses with the production of a wide range of inflammatory mediators, and consequently leading to mucosal damage^[34]. CD is regarded to be a Th1 immune reaction driven state, whereas UC is a Th2 immune state. Bacterial recognition is dependent on transmembrane pattern recognition receptors of intestinal epithelial cells, including toll-like receptors (TLR) and the intracellular NOD-like receptor family^[5,31,35,36]. Ligation of these bacterial receptors stimulates central signaling cascades that include the nuclear factor-kappaB (NF κ B) pathway, one of the key pathways in mucosal homeostasis that is shown to be elevated in the chronic inflammation tissue of the IBD^[5,37].

Composition of gut microbiota in patients with IBD has been extensively studied over the last decade. Although methodologies and results may differ, some gen-

eralizations are possible^[38]. Numerous studies revealed that fecal microbiota has a different composition in IBD patients compared to healthy controls, and some differences between microbial populations in CD and UC were found. Similar findings were described for mucosa-associated microbiota, a bacterial population present on the mucosal surface that is in direct interaction with intestinal epithelial and immune system cells^[39-42]. Moreover, differences were observed between active and non-active stages of the disease as well as between inflamed and non-inflamed regions of the intestine^[41,43,44]. When studying intestinal flora in IBD, it is important to keep in mind certain facts. Firstly, only up to 30% of the total microflora can be identified using conventional bacteriological techniques^[38], however using molecular techniques has greatly improved the detection rate, though significant numbers of bacteria can still be left undetected^[38,45]. Secondly, many strains found in IBD do not belong to major phylogenetic groups represented in healthy individuals^[38,46]. Furthermore, a distinction should be made between mucosal flora and fecal flora. The composition of these two domains is unique, which seems to be important in IBD^[38,47].

Concentrations of mucosal bacteria are high in patients with bowel inflammation, especially those with CD, whereas they are low in healthy controls. Bacterial invasion of mucosa was evident in up to 83% of biopsies from IBD patients, while no bacteria were detected in tissue samples from controls^[45,48]. Functional alterations are most evident in adherent, invasive *Escherichia coli* that colonize the ileum of Crohn's disease patients^[49]. Fluorescent in situ hybridization studies demonstrate dramatically increased mucosa-associated bacteria in active Crohn's disease, and to a lesser extent in ulcerative colitis^[48]. The fecal microbiota differs from the mucosa-associated microbiota^[6], with the latter probably being more relevant for intestinal immunomodulation^[48].

Reduced microbial diversity in inflammatory bowel disease has been previously reported^[50-52]. Ott *et al*^[50] demonstrated that mucosal inflammation in IBD was associated with a loss of normal anaerobic bacteria; the reduction in diversity in IBD was due to a significant loss of *Bacteroides*, *Eubacterium*, and *Lactobacillus* species. The reduction in mucosa-associated *Bifidobacteria* and increase in *E. coli* and *Clostridia* in patients with IBD supports the hypothesis that an imbalance between potentially beneficial and pathogenic bacteria may contribute to its pathogenesis^[50,53-55]. Manichanh *et al*^[52] used a metagenomic approach to demonstrate the reduced complexity of the bacterial phylum *Firmicutes*, in particular *Clostridium leptum*, in CD patients compared to healthy controls. In general, fewer *Bacteroidetes* and *Firmicutes* were found^[56,57], including *Faecalibacterium prausnitzii* and bacterial species with a large butyrate-generating and anti-inflammatory capacity^[39,42,57,58], as well as a reduced diversity within this phylum^[59]. The counts of other short chain fatty acid (SCFA) producing bacteria such as *Bifidobacteria* are also reduced and consequently concentrations of SCFA in the intes-

tine decrease^[60-62]. Other studies have shown, however, that the number of mucosa-associated bacteria increased with the increase of *Enterobacteriaceae*, including adherent-invasive *E. coli*^[43,62-64].

A comprehensive study of 190 resected tissue samples by Frank *et al* showed decreased numbers of the phyla *Firmicutes* and *Bacteroidetes* with concomitant increases in *Proteobacteria* and *Actinobacteria*^[59]. In a study of adult patients, Gophna *et al* compared the tissue-associated intestinal microbiota in biopsy samples from patients with CD and UC, as well as from healthy controls. Their findings showed a significant increase of *Proteobacteria* and *Bacteroidetes* in CD patients and a decrease in *Clostridia* in this group. Comparison between the ulcerative colitis and healthy control groups displayed no significant differences. Based on the finding that the microbiota was of similar composition in samples from inflamed and non-inflamed tissues within the same individual, they concluded that imbalance in microbiota in CD is probably not sufficient to cause inflammation^[64].

Nwosu *et al*^[65] investigated correlation of age dependency and IBD. Their findings demonstrated an apparent opposite age-related trend for *Bacteroides* and *Escherichia* between UC and CD, suggesting an immunological effect of *Bacteroides* on promoting CD at early age while later having a protective role, suggesting that these differences reflect underlying immunological disorders for CD and UC.

Up to 95% of patients with active colitis may harbor sulfate reducing bacteria (SRB)^[55,66,67]. Fecal samples of patients with UC have been shown to have greater than normal levels of SRB and it has been suggested that SRB may play an important role in UC pathogenesis. Theoretically, the impairment of butyrate metabolism within colonocytes may lead to increased villous atrophy, which is one of the features of active inflammation of colonic mucosa^[54].

Pediatric populations are useful for research into gut microbiota in IBD, as most pediatric patients are treatment-naïve or newly diagnosed. Although most research has been performed on adults, microbiota of pediatric IBD has been increasingly investigated over the last few years. The first larger pediatric microbiota investigation in IBD patients by Conte *et al*^[29] showed a higher number of mucosa-associated facultative-anaerobic and aerobic bacteria in the ileum, cecum, and rectum of children with IBD than in controls, with the highest numbers found in patients with indeterminate colitis and Crohn's disease. They also found a good deal of individual variability in the concentrations of mucosa-associated bacteria within the different groups of patients examined, although the highest heterogeneity of species was found in the ileal mucosa of patients with Crohn's disease.

Microbial dysbiosis was also demonstrated using fecal samples in 19 children with newly diagnosed Crohn's disease. This study showed significantly lower concentrations of *Firmicutes*, mainly due to changes in detection within the *Clostridia* class, and higher concentrations of *Proteobacteria* and *Bacteroidetes*, whereas the concentration

of *Actinobacteria* was similar in CD patients and controls. Furthermore, Kaakoush *et al*^[68] concluded that the ratio of *Bacteroidetes* to *Firmicutes* increased with the PCDAI activity index of the patients.

Lionetti *et al*^[69] suggested that a possible mechanism of action of enteral nutrition in inducing disease remission in pediatric patients with Crohn's disease is the modification capacity of the gut microbiota. This was supported by the findings that in 8 out of 9 pediatric Crohn's patients, enteral nutrition alone induced disease remission. In all children with CD, analysis of gel band distribution revealed profound modification of the fecal microflora after exclusive enteral nutrition therapy, whereas in healthy controls no modification of microflora was detected and a bacterial profile analysis remained stable during the 3-mo observation period.

Horizontal distribution of the fecal microbiota in adolescents with IBD was investigated by Gosiewski *et al*^[70], who demonstrated that distribution of the microbiota in the colon is layered. Their results demonstrated that the quantitative composition of the bacterial microbiota changed in the consecutive fecal fractions and tissue samples of patients with CD and UC, whereas in the control group there were no differences in microbiota composition in consecutive fecal and tissue samples. The largest differences in the total proportion of bacteria were visible in the *Bifidobacterium* genus, whose number declined with consecutive fractions, whereas in controls it remained high in all fractions. Also, in patients with CD, the percentage of bacteria from the *Streptococcus* genus and *Enterobacteriaceae* in subsequent fractions increased in comparison to the control group, and in patients with UC similar findings were described for *Lactobacilli*. Investigation of the *Bacteroides* spp. showed that their percentage dropped in the consecutive fecal fractions in CD, similarly to the control group, whereas in patients with UC it increased. Only in the UC group was the bacterial flora attached to the mucous layer found to exert degrading action on the protective mucin^[70]. Mucus layer thickness in adolescents with IBD was studied in a group by Fyderik *et al*^[71]. They demonstrated that the mucus layer in the inflamed sites was significantly thinner as compared to controls and to non-inflamed sites in IBD patients. Furthermore, they reported that *Streptococcus* spp. were predominant in the inflamed mucosa in CD patients, and *Lactobacilli* spp. were predominant in UC patients.

In a study of 15 treatment-naïve pediatric patients with CD and 26 healthy controls, Kellermayer *et al*^[72] investigated mucosal microbiota with high-throughput methodologies. Using distance-based redundancy analysis, they showed that there was significant separation between the CD-associated colonic mucosal microbiota and the microbiota of controls. They also showed that patients with granulomatous CD had a higher number of genera and species, significantly differentiating the colonic mucosal microbiota from controls and patients without granulomas. The most prominent genera distinguishing granulomatous CD from non-granulomatous were *Rumi-*

nococcus, *Roseburia*, *Eggerthella* (all three decreased), and *Porphyromonas* (increased). There was a trend for the genera *Faecalibacterium* to be decreased in the transverse colonic mucosa of granulomatous patients with CD compared with non-granulomatous disease^[72].

A Scottish group by Hansen *et al* has been intensively investigating pediatric gut microbiota in IBD patients over the last few years. They have reported differences in colonic mucosal bacteria between pediatric UC patients and controls. Contrary to findings from previous studies, they reported a reduction in *Bacteroidetes* and an increase in *Firmicutes*^[73]. They also described a reduction in bacterial diversity and an increased concentration of *Faecalibacterium prausnitzii* in de-novo pediatric CD patients, a finding contradicting the current protective role model of *F. prausnitzii* in CD^[74]. In the latest study by this group, microaerophilic microbiota of pediatric IBD onset has been researched. *Campylobacter* appears to be commonly isolated from pediatric colonic biopsies, but does not seem to be strongly associated with IBD. As a common commensal in pediatric gut microbiota, *Sutterella wadsworthensis* has also been reported^[75].

Despite many discoveries in the last two decades, it remains unknown whether the intestinal microbiota triggers and maintains the chronicity of inflammatory response in IBD, or is altered as a secondary response to intestinal inflammation^[76].

PROBIOTICS AND PREBIOTICS

Probiotics are specific live microorganisms which, when ingested in sufficient amounts, can promote health in the host^[77]. In order to qualify as probiotic, microorganisms must fulfill a number of criteria^[78]. They should be strictly specified at the genus, species, and strain levels, and specific strains should be registered and disposed in an international culture collection. Thus, generalizations concerning the efficacy of a whole species or even genus might be misleading. Probiotics should be extremely safe; their safety is supported by the fact that many strains are of human origin and have a long history of safe use. Many probiotics and their applications have been granted GRAS (generally regarded as safe) status. Although this classification should not be generalized, it does not warrant permanent surveillance for potential risks, such as invasiveness and potential for transfer of antibiotic resistance to other microorganisms^[79,80]. Because the effects of probiotic microorganisms are generally dependent on their viability, their stability during processing and storage, as well as their ability to survive intestinal transit through the stomach and proximal small bowel to finally adhere to mucosa and colonize the intestine, should be demonstrated^[78]. The final, but perhaps one of the most important, criteria for specific microorganism to be qualified as probiotic is a scientifically proven effect on the promotion of health or prevention and treatment of a specific disease^[78].

Prebiotics are non-digestible food ingredients that

selectively stimulate favorable bacterial growth and/or promote activity of a limited number of health-promoting bacteria, hence benefiting the host^[81,82]. However, prebiotics can also be applied to enhance the survival and action of ingested probiotic bacteria. When probiotics and prebiotics are combined in one product to achieve synergistic effects they are usually called synbiotics. The vast majority of prebiotic substances are carbohydrates that are indigestible for human digestive enzymes but can be fermented by beneficial bacterial genera in the colon and serve as a substrate for their metabolism. Some of them can be found in natural foods, such as human milk oligosaccharides in mother's milk, while others are added to food. Good examples of prebiotics are fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), soybean oligosaccharides, and complex polysaccharides that constitute dietary fiber^[81].

Probiotics or prebiotics may achieve their therapeutic effect in IBD through many different mechanisms. They influence the composition of intestinal microbiota and alter the metabolic properties of the microbiome^[76]. By increasing the production of short-chain fatty acids, they may lower the pH of the colonic environment and thus inhibit the growth of potentially pathogenic microorganisms. Butyrate plays a trophic role as a nutrient for colonocytes and enhances repair of injured gut epithelium in IBD. Moreover, evidence shows that butyrate acts directly as an anti-inflammatory agent by inactivating the intracellular transcriptional factor NF- κ B pathway, consequently attenuating synthesis of inflammatory cytokines^[8]. A large number of probiotic strains are able to produce antibacterial substances, such as hydrogen peroxide, hydrogen sulfide, lactic acid, and specific bacteriocins^[83], as well as displace deleterious microbes from the luminal-mucosal interface by competing for binding sites on the epithelial cell surface or mucus layer^[84,85].

Probiotics communicate with epithelial cells and different sets of cells implicated in both innate and acquired immune response via pattern-recognition receptors^[3]. They can enhance gut barrier function and reduce intestinal permeability for intestinal microorganisms and other antigens^[86]. For example, several strains of *Lactobacilli* can up-regulate MUC3 gene expression, resulting in increased mucus production by intestinal goblet cells^[87,88]. Several probiotic strains can induce the production and secretion of different anti-microbial peptides by epithelial cells, such as defensins, lysozyme, lactoferrin, or phospholipase, and directly decrease permeability of the epithelial layer by enhancing tight junctions and reducing epithelial cell apoptosis^[85,89,90].

Each probiotic strain may have distinct immunoregulatory properties, thus probiotics can indirectly or directly modulate intestinal immune response. In very simplified terms, probiotics can be classified into two groups with regards to their influence on the immune system: one exhibiting immunostimulating activities and the other anti-inflammatory properties^[91]. Numerous studies have revealed the mechanisms by which probiotics down-reg-

ulate the inflammatory immune response, including those with proven clinical efficacy in the therapy of IBD. Some probiotic strains may induce maturation of intestinal dendritic cells, an important part of antigen presenting and immune regulation, and extend their survival^[92]. Several probiotics act through strengthening the regulatory T cell (Treg) response. Tregs are antigen-specific T cells which prevent autoimmunity and preserve tolerance towards harmless antigens, including intestinal commensal microbiota^[84]. They can control excessive NF κ B pathway activation, decrease production of pro-inflammatory cytokines (*e.g.*, TNF α , INF γ , and IL-8), and induce the production and secretion of anti-inflammatory cytokines such as IL-10 and TGF β ^[3,91,93,94].

It is possible that there are further mechanisms of probiotic action that have not yet been demonstrated. Regarding the fact that pathogenesis of each type of IBD differs and that mechanisms of action of probiotics are strain-specific and very different, we might expect that different probiotics would be effective for each type and phase of the disease.

Over the last two decades, several interventional clinical studies comparing the efficacy of probiotic therapy against placebo or standard therapy with drugs have been published. The use of different study designs (*e.g.*, concomitant use of other forms of therapy) and various probiotic strains and doses, with only a few studies resembling one another in such a manner to be able to uniformly compare the results, makes it very difficult to derive any firm conclusions.

TREATMENT OF ACTIVE ULCERATIVE COLITIS

Clinical studies on the efficacy of probiotics for the induction of remission in ulcerative colitis gave encouraging, albeit conflicting, results. Bennet and Brinkman first reported a successful induction of long-lasting remission by a single enema of the fecal microbiota of a healthy donor in a patient with active UC^[95]. Borody *et al*^[96] published six cases of patients with UC resistant to medical therapy with steroids and immunomodulators who underwent transplantation of fecal microbiota from healthy donors by repeated enemas after 7-10 d of pre-therapy with vancomycin, metronidazole, rifampicin, and bowel lavage with polyethylene glycol. Complete reversal of UC was achieved in all patients, and they were all able to stop anti-inflammatory therapy after 6 wk. After 1 to 13 years of follow-up, all patients remained in complete clinical, endoscopic, and histologic remission without any adjunctive therapy.

Several studies investigated the efficacy of multispecies probiotic VSL#3 containing four strains of *Lactobacilli* (*L. casei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii* subsp. *bulgaricus*), three strains of *Bifidobacteria* (*B. longum*, *B. breve*, and *B. infantis*) and one strain of *Streptococcus* (*S. salivarius* subsp. *thermophilus*). Tursi *et al*^[97] compared the efficacy and safety of low-dose balsalazide (2.25 g/d)

plus 3 g/d VSL#3 (group A, *n* = 30), with medium-dose balsalazide alone (group B, *n* = 30), and with mesalazine (group C, *n* = 30) in the 8-wk treatment of mild to moderate active ulcerative colitis. Efficacy was assessed by assessment of symptoms, endoscopic appearance, and histological evaluation. Balsalazide/VSL#3 was significantly superior to balsalazide alone and to mesalazine in obtaining remission (85.71% *vs* 80.77% *vs* 72.73%, respectively; *P* < 0.02). The balsalazide/VSL#3 combination was faster in obtaining remission than balsalazide alone or mesalazine (4, 7.5, and 13 d, respectively), and was also better in improving all parameters evaluated. Moreover, balsalazide with or without VSL#3 was better tolerated than mesalazine. The authors concluded that balsalazide/VSL#3 might be a very good choice in the treatment of active mild-to-moderate active ulcerative colitis. Bibiloni *et al*^[98] studied the efficacy and safety of VSL#3 for induction of remission in an open-label study in 34 ambulatory patients with mild to moderate active UC. Among 32 patients who completed 6-wk treatment with VSL#3 3.6×10^9 CFU/d, remission (defined as UCDAI \leq 2) was achieved in 53% and response (decrease in UCDAI \geq 3, but final score \geq 3) in 24%. In 9% of patients there was no response, in another 9% worsening of the condition was observed, and in 5% there was no final endoscopic assessment. The investigators reported no biochemical or clinical adverse events related to VSL#3. In addition, they confirmed the presence of VSL#3 species by DNA sequencing of 16S rRNA in biopsies collected from patients in remission. A small open-label pilot study on 18 pediatric patients between the ages of 3-17 years with mild to moderate acute UC using VSL#3 for 8 wk was performed by Huynh *et al*^[99]. The simple clinical colitis activity index (SCCAI) was used to assess disease activity. Remission (defined as SCCAI \leq 3) was achieved in 56% and response (decrease in SCCAI \geq 2, but final score \leq 5) in 6%, with no change or worsening reported in 39% of patients. Five patients were withdrawn due to lack of improvement and only 13 patients completed 8 wk of VSL#3 treatment. VSL#3 was well tolerated, and no biochemical or clinical adverse effects attributed to VSL#3 were identified.

Tursi *et al*^[100] compared the efficacy of VSL#3 in a dosage of 3.6×10^9 CFU (*n* = 65) with placebo (*n* = 66) in achieving remission in UC patients on concomitant therapy with aminosalicylates and/or immunosuppressants. After 8 wk of treatment, the decrease in UCDAI of 50% or more was significantly higher in the VSL#3 group (63.1%) than in the placebo group (40.8%) (*P* = 0.010). A decrease of three points or more in the UCDAI score was achieved in 60.5% in the VSL#3 group *vs* 41.4% in the placebo group (*P* = 0.017). They also found a significant difference in rectal bleeding (*P* = 0.014) but not in stool frequency, physician's rate of disease activity, or endoscopic score. Remission was slightly higher in the VSL#3 group than in the placebo group (47.7% *vs* 32.4%; *P* = 0.069).

In a randomized, multicenter, double-blind, controlled

trial, Sood *et al.*^[101] compared the efficacy of VSL#3 applied twice daily in a dosage of 3.6×10^9 CFU ($n = 77$) to placebo ($n = 70$) for induction of remission of mild to moderate UC. The primary endpoint was a 50% decrease in the ulcerative colitis disease activity index (UCDAI) at 6 wk. The secondary endpoints included remission by 12 wk and reduction in total individual UCDAI parameters from baseline at 12 wk. At week 6, the percentage of patients with an improvement in UCDAI score that was greater than 50% was significantly higher in the group given VSL#3 (32.5%) than the group given placebo (10%) ($P = 0.001$). At week 12, 42.9% patients given VSL#3 achieved remission, compared with only 15.7% patients given placebo ($P < 0.001$). Furthermore, significantly more patients given VSL#3 (51.9%) achieved a decrease in their UCDAI that was greater than 3 points, compared with those given placebo (18.6%) ($P < 0.001$). The VSL#3 group had significantly greater decreases in UCDAI scores and individual symptoms at weeks 6 and 12 compared with the placebo group.

Miele *et al.*^[102] performed a 1-year prospective, placebo-controlled, double-blind pediatric study to assess the efficacy of VSL#3 on the induction and maintenance of remission in children with active UC. A total of 29 consecutive patients (mean age: 9.8 years; range: 1.7-16.1 years) with newly diagnosed UC were randomized to receive either a weight-based dose of VSL#3 ($n = 14$) or placebo ($n = 15$) in conjunction with concomitant steroid induction and mesalamine maintenance treatment. The Lichtiger colitis activity index and a physician's global assessment were used to measure disease activity. At baseline (within 6 mo, 12 mo, or at the time of relapse), all patients were assessed endoscopically and histologically. All 29 patients responded to the induction therapy. Remission was achieved in 92.8% children treated with VSL#3 and standard therapy compared to only 36.4% treated with placebo and standard therapy ($P < 0.001$). Moreover, only 21.4% patients treated with VSL#3 relapsed within 1 year of follow-up compared to 73.3% patients from the placebo group ($P = 0.014$). At 6 mo, 12 mo, or at time of relapse, endoscopic and histological scores were significantly lower in the VSL#3 group than in the placebo group ($P < 0.05$). There were no biochemical or clinical adverse events related to VSL#3. This study demonstrated the efficacy of VSL#3 both in the induction and maintenance of remission in pediatric UC patients.

In a small open-label study by Tsuda *et al.*^[103], the effectiveness of another multispecies probiotic preparation BIO-THREE (containing *Streptococcus faecalis*, *Clostridium butyricum*, and *Bacillus mesentericus*) was tested for treatment of mild to moderate distal UC refractory to conventional therapies. Twenty patients were treated for 4 wk. Clinical symptoms and endoscopic findings were evaluated, and UCDAI scores calculated before and after treatment. In addition, fecal microbiota was analyzed by the terminal restriction fragment length polymorphism (T-RFLP) method. Remission (UCDAI score ≤ 2) was observed

in 45% and response (decrease in UCDAI ≥ 3 , but final score ≥ 3) in 10%, however in 40% there was no response and in 5% they found worsening (UCDAI > 3) of the disease. T-RFLP analysis indicated an increase in *Bifidobacteria*.

In a single-center, randomized, double-dummy study, Rembacken *et al.*^[104] examined whether the addition of a non-pathogenic strain of *E. coli* Nissle 1917 to standard medical therapy increased the chance of remission of active ulcerative colitis and whether this probiotic strain was as effective as mesalazine in preventing relapse. Of a total of 116 patients, 59 were randomized to the mesalazine group and 57 to the *E. coli* group. All patients received concomitant standard medical therapy with tapering steroids together with a 1-wk course of oral gentamicin. After remission, patients were maintained on either mesalazine or *E. coli*, and followed-up for 1 year. The investigators found no significant differences between the mesalazine and *E. coli* groups in percentage of patients that achieved remission, mean time to remission, percentage of patients who relapsed, and mean duration of remission. Although the addition of *E. coli* to standard therapy did not increase the induction rate of remission, the results suggested that treatment with this probiotic might have an equivalent effect to mesalazine in maintaining remission of ulcerative colitis.

Kato *et al.*^[105] conducted a randomized placebo-controlled trial using *Bifidobacteria*-fermented milk (BFM) (containing *Bifidobacterium breve* strain Yakult, *B. bifidum*, and *Lactobacillus acidophilus*) supplementation as a dietary adjunct in treating active ulcerative colitis. Twenty patients with mild to moderate active UC randomly received 100 mL/d of BFM or placebo for 12 wk with conventional treatment. The clinical activity index was significantly lower in the BFM than in the placebo group, and the endoscopic activity index and histological score were significantly reduced in the BFM, but not the placebo group, after treatment. They also observed an increase in fecal butyrate, propionate, and short-chain fatty acid concentrations in the BFM, but not the placebo group. Therefore, the authors concluded that supplementation with this *Bifidobacteria*-fermented milk product is safer and more effective than conventional treatment of active UC alone.

Ishikawa *et al.*^[106] compared a group of patients with BFM supplementation 100 mL/d ($n = 11$) and a control group ($n = 10$), both receiving standard medical treatment of ulcerative colitis. Colonoscopies, general blood markers, and examinations of intestinal flora, including the analysis of fecal organic acids, were performed at the initiation of the study and after one year. Exacerbation of symptoms was observed in 3 out of 11 subjects in the BFM group and in 9 out of 10 in the control group. Statistical analysis of the cumulative exacerbation rates showed a significant reduction in exacerbations for the BFM group ($P = 0.0184$). A significant reduction in the relative proportion of *B. vulgatus* in *Bacteroidaceae* and butyrate concentration was observed after supplementation

with BFM in comparison with before.

Recently, Oliva *et al*^[107] published a prospective, randomized, placebo-controlled study comparing the effectiveness of *Lactobacillus reuteri* ATCC 55730 enema and placebo in children with active distal UC. A total of 40 patients (median age 7.2 years; range 6-18 years) were enrolled. They received an enema solution containing 10^{10} CFU of *L. reuteri* or placebo for 8 wk, in addition to oral mesalazine. Clinical, endoscopic, and histological scores, as well as rectal mucosal expression levels of pro- and anti-inflammatory cytokines, were evaluated at the beginning and at the end of the trial. Mayo score (including clinical and endoscopic features) as well as histological score decreased significantly in the *L. reuteri* group ($P < 0.01$), but not in the placebo group. Moreover, the evaluation of cytokine mucosal expression levels revealed that IL-10 significantly increased ($P < 0.01$), whereas IL-1 β , TNF α , and IL-8 significantly decreased ($P < 0.01$) only in the *L. reuteri* group.

In a small non-controlled pilot study, Guslandi *et al*^[108] treated 25 patients with mild to moderate clinical flare-up of ulcerative colitis with *Saccharomyces boulardii* 250 mg three times a day for 4 wk during maintenance treatment with mesalazine. Of the 24 patients who completed the study, 17 attained clinical and endoscopic remission.

Furrie *et al*^[109] explored the efficacy of a synbiotic combining a probiotic strain of *Bifidobacterium longum* and a prebiotic (Synergy 1), a preferential inulin-oligofructose growth substrate for this probiotic strain. Treatment was used in a double-blinded randomized controlled trial in 18 patients with active UC for a period of one month. Although the subsequent sigmoidoscopy score decrease in the synbiotic group was not statistically significant compared with placebo ($P = 0.06$), they found that biopsies in the test group had reduced inflammation, and increased regeneration of epithelial tissue and mRNA levels for beta defensins 2, 3 and 4 (which are strongly up-regulated in active UC), tumor necrosis factor alpha and interleukin-1 alpha were also significantly reduced in the test group after treatment ($P = 0.016, 0.038, 0.008, 0.018$ and 0.023 , respectively).

In another study by Ishikawa *et al*^[110], the investigators examined the effects of a live *Bifidobacterium breve* strain Yakult and GOS as synbiotic in active UC. Forty-one patients with mild to moderate UC were assigned to two groups; one was treated with the synbiotic (1 g of the probiotic powder (10^9 CFU/g) three times a day and 5.5 g of GOS once a day) and the other was not (control group). After one-year treatment with the synbiotic, the clinical status of the UC patients as assessed by colonoscopy significantly improved, and the amount of myeloperoxidase in the lavage, a marker of inflammation, decreased. The synbiotic also significantly reduced the fecal counts of *Bacteroidaceae* and fecal pH.

Several reviews and meta-analyses have been performed over recent years concerning the induction of remission in ulcerative colitis by probiotics. In a Cochrane Collaboration review from 2007, the authors as-

sessed the efficacy of probiotics compared to placebo or standard medical treatment with 5-aminosalicylates, sulfasalazine, or corticosteroids^[111]. Only 4 randomized controlled trials met the criteria, and a formal meta-analysis could not be performed because of heterogeneity in methodology, probiotic strains, and outcomes. The authors concluded that combining conventional therapy with probiotics did not improve overall remission rates in patients with mild to moderate UC. However, they found limited evidence that the addition of probiotics might provide modest benefits in terms of disease activity. The negativistic opinion shared in this early review can be at least partially attributed to the low number of high quality studies published at the time. In a meta-analysis later performed by Sang *et al*^[112] and published in 2010, both the induction of remission and maintenance were compared between probiotic and non-probiotic treatment in ulcerative colitis. Thirteen randomized controlled studies met the selection criteria. Seven studies evaluated the remission rate, 8 the recurrence rate, and 2 both remission and recurrence rates. The remission rate for probiotics compared with non-probiotics therapy was 1.35 (95%CI: 0.98-1.85), while when compared with the placebo it was 2.00 (95%CI: 1.35-2.96). Although these differences were not statistically significant, the authors concluded that these results were probably subject to heterogeneous bias. Regarding maintenance of remission, the recurrence rate of ulcerative colitis in patients who received probiotics was 0.69 (95%CI: 0.47-1.01) and 0.25 (95%CI: 0.12-0.51) in patients with mild to moderate UC compared with the non-probiotic group. The group who received *Bifidobacterium bifidum* treatment had a recurrence rate of 0.25 (95%CI: 0.12-0.50) compared with the non-probiotics group. The authors concluded that probiotic treatment was more effective than placebo in maintaining remission in ulcerative colitis.

In contrast with these reviews, a meta-analysis performed by Zigra *et al*^[113] showed a significant benefit of probiotic use for UC remission induction with pooled relative risk 2.27 (95%CI: 1.00-5.14, $P = 0.049$).

In a more recent review by Jonkers *et al*^[56], only subgroup-specific meta-analyses per probiotic were performed. The only probiotic with several published randomized controlled studies for induction of remission in adult patients with UC was VSL#3. The calculated pooled RR for VSL#3 was 1.69 (95%CI: 1.17-2.43), indicating a significant benefit of VSL#3 over control in inducing remission in active UC.

Interestingly, in the 2011 recommendations for probiotic use from the 3rd Yale Workshop, both VSL#3 and *Escherichia coli* Nissle 1017 were rated B, meaning that recommendation of their use for induction of remission in UC is based on positive controlled studies, but with the presence of some negative studies that did not support the primary outcome^[114].

In conclusion, the results of several clinical studies suggest that the addition of specific probiotics to conventional therapy in active UC may be beneficial. The

strongest evidence exists for multispecies preparation VSL#3, with several studies both in adults and children supporting its efficacy.

MAINTENANCE OF REMISSION IN ULCERATIVE COLITIS

There have been several published studies in which efficacy of the probiotic strain of *Escherichia coli* Nissle 1917 was compared to either placebo or standard therapy for maintenance therapy in UC. In a double-blind, double-dummy study by Kruis *et al.*^[115], 120 patients with inactive ulcerative colitis were randomized to mesalazine 500 mg three times daily or to an oral preparation of *E. coli* Nissle treatment for 12 wk to compare their efficacy in preventing a relapse of the disease. Study objectives were to assess the equivalence of the two therapeutic modalities by comparing the clinical activity index (CAI), relapse rates, relapse-free times, and global assessment. The start and end CAI scores demonstrated no significant difference ($P = 0.12$) between the two treatment groups. Relapse rates were 11.3% under mesalazine and 16.0% under *E. coli* (N.S.), and the relapse-free time was similar for mesalazine and *E. coli* (103 \pm 4 d and 106 \pm 5 d, respectively). Global assessment was also similar for both groups. Tolerability of the treatment was excellent in both groups. Conclusions of this study were that probiotic treatment with *E. coli* Nissle 1917 offered another option for maintenance therapy of ulcerative colitis. Subsequently, the same group performed another, albeit larger, double-blind, double dummy trial to confirm the equivalent efficacy of *Escherichia coli* Nissle 1917 and mesalazine in the maintenance of remission in UC^[116]. Patients received either the probiotic drug 200 mg once daily ($n = 162$) or mesalazine 500 mg three times daily ($n = 165$) for 12 mo, and were assessed by clinical and endoscopic activity indices (Rachmilewitz) as well as by histology. The per-protocol analysis revealed relapses in 40/110 (36.4%) patients in the *E. coli* group and 38/112 (33.9%) in the mesalazine group (significant equivalence $P = 0.003$). Subgroup analyses showed no differences between the treatment groups in terms of duration and localization of disease or pretrial treatment. Safety profile and tolerability were very good for both groups. By the end of this second study the authors concluded that *E. coli* Nissle 1917 showed the same equivalent efficacy and safety as mesalazine in maintaining remission in patients with ulcerative colitis.

In another trial by Rembacken *et al.*^[104], both the capacity of induction and maintenance of remission by *E. coli* Nissle 1917 were evaluated. In this single-center, randomized, double-dummy study, patients were maintained on either mesalazine ($n = 59$) or *E. coli* ($n = 57$) and followed up for a maximum of 12 mo. A comparable percentage of patients relapsed in the mesalazine (73%) and *E. coli* groups (67%), and the mean duration of remission was practically similar in both (206 and 221 d, respectively). Again, the authors came to the conclusion

that treatment with non-pathogenic *E. coli* was as equivalently efficient as mesalazine in maintaining remission of ulcerative colitis.

Zocco *et al.*^[117] studied the efficacy of a probiotic strain of *Lactobacillus rhamnosus* GG for maintenance therapy in UC. They randomized patients into three groups: *Lactobacillus* GG 18×10^9 CFU/d ($n = 65$), mesalazine 2400 mg/d ($n = 60$), or a combination of *Lactobacillus* GG and mesalazine ($n = 62$). Overall analysis of UCDAI scores and endoscopy and histology results showed no difference in relapse rate at 6 and 12 mo among the three groups. However, treatment with *Lactobacillus* GG alone or in combination seemed to be more effective than standard treatment with mesalazine in prolonging relapse-free time ($P < 0.05$).

A non-controlled trial using multispecies preparation VSL#3 in 20 UC patients in remission, intolerant, or allergic to 5-aminosalicylates for 12 mo was performed by Venturi *et al.*^[118]. They reported that 15 out of 20 patients remained in remission during the study, 4 relapsed, and one was lost to follow-up. They suggested that VSL#3 might be useful in maintaining remission in UC patients intolerant to standard therapy.

In the previously mentioned pediatric study by Miele *et al.*^[102], the investigators observed that only 21.4% of patients treated with VSL#3 (compared to 73.3% patients from the placebo group) relapsed within 1 year of follow-up ($P = 0.014$). They also found significantly lower endoscopic and histological scores in the VSL#3 group than in the placebo group ($P < 0.05$). The results of this study confirmed the efficacy of VSL#3 in the maintenance of remission in pediatric UC patients.

Cui *et al.*^[119] randomized 30 patients with UC in remission achieved by treatment with sulfasalazine and glucocorticoids into two groups: one that received bifid triple viable capsule (BIFICO) (1.26 g/d) for 8 wk and the other an identical placebo group. The patients were evaluated clinically, endoscopically, and histologically after 2 mo of treatment or in the event of UC relapse. Only three patients (20%) in the BIFICO group relapsed during the 2-mo follow-up period compared with 14 (93.3%) in the placebo group ($P < 0.01$). Moreover, the microbiological and immunological analyses revealed that the concentration of fecal *Lactobacilli* and *Bifidobacteria* was significantly increased only in the BIFICO-treated group ($P < 0.01$). The expression of pro-inflammatory NF κ B p65 and DNA binding activity of NF κ B were significantly attenuated, and the mRNA expression of anti-inflammatory cytokines was elevated in the treatment group in comparison with the control group ($P < 0.05$). The authors concluded that oral administration of probiotic preparation BIFICO was effective in preventing flare-ups of chronic UC.

Shanahan *et al.*^[120] performed a double-blind, placebo-controlled study on 157 patients to compare the efficacy of *Lactobacillus salivarius* subspecies *salivarius* UCC118, *Bifidobacterium infantis* 35624 (1×10^9 CFU/d), or placebo for maintenance UC therapy. They found no difference

in relapse time between probiotics and placebo.

Wildt *et al*^[121] performed a small double-blind placebo-controlled study using probiotic preparation Probio-Tec-AB-25 (containing the two probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subspecies *lactis* BB-12) or placebo in patients with left-sided UC in remission for 52 wk. 25% of patients on probiotics and 8% of those on placebo maintained remission after 1 year of treatment ($P = 0.37$). The median time to relapse was 125 d in the probiotic group and 104 d in the placebo group ($P = 0.683$). The authors concluded that no significant clinical benefit of Probio-Tec-AB-25 in comparison with placebo for maintaining remission in UC was demonstrated.

In the recent Cochrane Collaboration review of probiotic efficacy and safety for the maintenance of remission in UC by Naidoo *et al*^[122], only 4 studies met the inclusion criteria. Three of those trials compared probiotics to mesalazine, and one to placebo. The pooled analysis was performed and revealed no statistically significant differences in the efficacy of probiotics over mesalazine. Relapse was reported in 40.1% of patients treated with probiotics and in 34.1% of those on mesalazine therapy. No statistical difference in the incidence of adverse events between the two groups was demonstrated. In only one placebo-controlled study was the relapse rate between probiotic and placebo groups considered non-significant. The authors concluded that, given the relatively small number of patients included in the clinical studies, the evidence was insufficient to make conclusions about the efficacy of probiotics for the maintenance of remission in UC.

A subgroup probiotic-specific meta-analysis by Jonkers *et al*^[56] revealed that pooled relative risk for *E. coli* Nissle compared to mesalazine was 1.08 (95% CI 0.86-1.37), indicating that this strain of *E. coli* was not inferior to mesalazine in preventing relapses.

The American Recommendations for probiotic use from 2011 state very strong “A” recommendations for the use of the two specific probiotics *Escherichia coli* Nissle 1917 and multispecies mixture VSL#3 for the maintenance of remission in UC^[114].

In conclusion, specific probiotics such as *Escherichia coli* Nissle 1917 and multispecies mixture VSL#3 are probably as efficient as standard maintenance therapy with mesalazine, and can therefore be used instead of mesalazine in patients intolerant or allergic to 5-amino-salicylates, or as adjunctive therapy to standard therapy, to potentially increase the duration of remission.

TREATMENT AND PREVENTION OF POUCHITIS

In some patients with UC in whom the disease does not respond to medical therapy or who develop dysplasia or cancer, proctocolectomy with the construction of ileal pouch-anal anastomosis (IPAA) is required. Inflammation of this ileal reservoir (pouch), referred to as pouchitis, develops in between 15% and 50% of such patients.

Although the exact etiology of pouchitis is not clear, host genetic factors, fecal stasis, mucosal ischemia, and bacterial dysbiosis in the pouch seem to be involved^[56,87]. Most patients develop pouchitis in the first year after the procedure. Antibiotic therapy is generally successful; however, discontinuation of antibiotics is often followed by recurrence of the disease. Treatment and prevention of pouchitis with probiotics has thus been studied extensively, and only a few studies addressing the use of probiotics for the treatment of active pouchitis were published.

Kuisma *et al*^[123] performed a double-blind placebo-controlled trial to investigate the efficacy of *Lactobacillus rhamnosus* GG supplementation as primary therapy for ileal pouch inflammation. Twenty patients with a previous history of pouchitis and endoscopic evidence of inflammation were randomized to *Lactobacillus* GG $0.5-1 \times 10^{10}$ CFU twice daily or placebo for 3 mo. Clinical efficacy was assessed by a change in the pouchitis disease activity index (PDAI). In addition, quantitative bacterial cultures of fecal samples and biopsies taken from the pouch were performed before and after probiotic supplementation. No differences were observed between the groups with regard to the mean pouchitis disease activity index. *Lactobacillus* GG supplementation changed the pouch intestinal microbiota by increasing the ratio of total fecal *Lactobacilli* to total fecal anaerobes ($P = 0.03$) and enhancing the frequency of *Lactobacilli*-positive cultures in the pouch. The authors concluded that although probiotic supplementation with *Lactobacillus* GG changed pouch microbiota, it was clinically ineffective as primary therapy for active pouchitis.

In an open-label study, Laake *et al*^[124] treated 51 UC patients with IPAA, 6 UC patients with ileorectal anastomosis without pouch, and 10 patients with IPPA because of familial adenomatous polyposis with a fermented milk product culture, containing probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subspecies *lactis* BB-12, in a dosage of 5×10^{10} CFU/d for 4 wk. Stool samples were cultured for examination of *Lactobacilli*, *Bifidobacteriae*, fungi, and pH before, during, and after intervention. In addition, before, during, and after intervention, symptom assessment and endoscopic evaluation was performed. Symptoms, such as involuntary defecation, leakage, abdominal cramps, fecal number and consistency, mucus, and urge to evacuate stools were significantly decreased during intervention in the UC/IPAA group. The median endoscopic score of inflammation also significantly decreased. The number of *Lactobacilli* and *Bifidobacteriae* significantly increased during intervention and remained significantly increased one week after intervention.

Gionchetti *et al*^[125] evaluated the efficacy of high-dose VSL#3 in the treatment of mild active pouchitis in an open-label non-controlled study. Twenty-three patients with mild pouchitis were treated with VSL#3 (3.6×10^9 CFU/d) for four weeks. Symptomatic, endoscopic, and histologic evaluations were undertaken before and after treatment according to PDAI. Remission was defined as

a combination of a PDAI clinical score of ≤ 2 , an endoscopic score of ≤ 1 , and a total PDAI score of ≤ 4 . Patients in remission after initial treatment were treated with a maintenance dose of VSL#3 (1.8×10^9 CFU/d) for an additional six months. Sixteen out of 23 patients (69%) were in remission after treatment. The median total PDAI scores before and after therapy were 10 (range, 9-12) and 4 (range, 2-11), respectively ($P < 0.01$). The median Inflammatory Bowel Disease Questionnaire score also significantly improved ($P < 0.001$). All 16 patients who went into remission maintained remission during maintenance treatment. The authors conclude that high doses of the probiotic VSL#3 were effective in the treatment of mild pouchitis. As pouchitis is a recurrent state, many studies evaluated the potential of probiotics in preventing exacerbations. The most profoundly studied probiotic for this indication was multispecies preparation VSL#3. In a randomized, double-blind, placebo-controlled trial, the same group evaluated the efficacy of VSL#3 in the remission maintenance of chronic pouchitis compared with placebo^[126]. Forty patients in clinical and endoscopic remission achieved by antibiotic therapy were randomized to receive either VSL#3 3×10^{12} CFU/d or placebo for 9 mo. Patients were assessed clinically every month, and endoscopically and histologically every 2 mo or in the event of relapse. In addition, bacterial stool cultures from fecal samples were performed before and after antibiotic treatment and each month during maintenance treatment. Only 3 patients (15%) in the VSL#3 group had relapses within the 9-mo follow-up period, in comparison with 20 (100%) in the placebo group ($P < 0.001$). In the VSL#3-treated group (but not in the control group), fecal concentrations of *Lactobacilli*, *Bifidobacteria*, and *S. thermophilus* increased significantly from baseline levels during treatment ($P < 0.01$). Therefore, the authors concluded that oral administration of VSL#3 is effective in preventing flare-ups of chronic pouchitis.

In another double-blind, placebo-controlled study, Gionchetti *et al.*^[127] evaluated the effectiveness of VSL#3 therapy in preventing the onset of pouchitis immediately and during the first year after ileal pouch-anal anastomosis. For this purpose, 40 patients who underwent IPAA for UC were randomized to receive either VSL#3 9×10^{11} CFU/d ($n = 20$) or placebo ($n = 20$) immediately after ileostomy closure for 1 year. The patients were assessed clinically, endoscopically, and histologically every few months, and health-related quality of life was assessed using the Inflammatory Bowel Disease Questionnaire (IBDQ). Only 2 (10%) patients from the VSL#3 group, compared to 8 (40%) from the placebo group, had an episode of acute pouchitis (log-rank test, $Z = 2.273$; $P < 0.05$). As expected, treatment with VSL#3 (but not placebo) produced a significant improvement in IBDQ score.

In a study by Mimura *et al.*^[128], the researchers evaluated the effectiveness of a single daily high dose probiotic preparation of VSL#3 in maintaining antibiotic-induced remission. All patients included in this study had pouchitis

at least twice in the previous year or required treatment with continuous antibiotics. After remission was induced within four weeks of combined metronidazole and ciprofloxacin therapy, the patients were randomized to receive either VSL#3 (9×10^{11} CFU) ($n = 20$) or placebo ($n = 16$) once daily for one year or until relapse. Symptomatic, endoscopic, and histological evaluations were made before and 2 and 12 mo after randomization or at the time of relapse. Remission was maintained for one year in 17 patients (85%) on VSL#3, but in only one (6%) on placebo ($P < 0.0001$). The IBDQ score remained high in the VSL#3 group but deteriorated in the placebo group ($P = 0.0005$). Therefore, the authors concluded that the once daily high dose probiotic VSL#3 was effective in maintaining antibiotic introduced remission in patients with recurrent or refractory pouchitis.

In an open-label trial by Pronio *et al.*^[129], 31 patients at different periods after surgery without signs or symptoms of pouchitis were randomized to VSL#3 9×10^{11} CFU/d or no treatment for 12 mo. Pouchitis activity was evaluated by PDAI, with different immunologic parameters being studied in peripheral-blood mononuclear cells and mucosal biopsies to reveal the mechanisms of probiotic action. During the study period, none of the patients from the probiotic group and only one from the placebo group developed active pouchitis. Because of the extremely low relapse-rate, even in the non-treated group, it was impossible to derive any firm conclusions regarding the efficacy of probiotic treatment from this study. However, a significant reduction in PDAI score was observed in VSL#3 treated patients.

In contrast with these studies, Shen *et al.*^[130] reported much more disappointing results. In an open-label uncontrolled trial, they gave VSL#3 9×10^{11} CFU/d for 8 mo to 31 patients after being treated for pouchitis with ciprofloxacin for 2 wk. Baseline PDAI scores were calculated and patient symptoms were reassessed at week 3 of VSL#3 therapy and at the end of the 8-mo trial. Some, but not all, patients underwent repeat pouch endoscopy at the end of the trial. At the 8-mo follow-up, only 6 patients were still on VSL#3 therapy while all others had discontinued the therapy due to either recurrence of symptoms ($n = 23$) or development of adverse effects ($n = 2$). All six patients who completed the 8-mo course had repeat clinical and endoscopic evaluation. Their mean PDAI scores were not statistically different to those before probiotic intervention ($P = 0.27$). However, this trial had several methodological drawbacks. The patients were pre-treated with only one antibiotic and the success of this therapy of acute pouchitis was not regularly evaluated by endoscopy. Therefore, it remains unclear whether all patients were really in remission before the start of maintenance therapy with VSL#3.

In an open-label study by Gosselink *et al.*^[131], 39 patients given a fermented milk product containing *Lactobacillus rhamnosus* GG in a dosage of $1-2 \times 10^{10}$ CFU immediately after IPAA operation were compared to 78 patients without any maintenance treatment. The first

episodes of pouchitis were observed significantly less frequently in the *Lactobacillus* GG group than in the untreated group (cumulative risk at 3 years: 7% *vs* 29%, $P = 0.011$). Therefore, the authors concluded that daily intake of the fermented product containing *Lactobacillus* GG provided significant clinical benefits without side effects, and recommended its use for the primary prevention of pouchitis.

In the Cochrane Collaboration review by Holubar *et al*^[132] published in 2010, different modalities for the treatment and prevention of pouchitis after ileal pouch-anal anastomosis for UC, including different antibiotics, probiotics, glutamine, butyrate, and budesonide, were meta-analyzed and reviewed. They concluded that *Lactobacillus* GG was not superior in effectiveness compared to placebo for the treatment of acute pouchitis, while VSL#3 was more effective than placebo in the maintenance therapy of chronic pouchitis (97% *vs* 3%, $P < 0.0001$). The number needed to treat with VSL#3 to prevent one additional relapse was 2. Similarly, in a strain-specific meta-analysis performed by Jonkers *et al*^[56], the authors calculated the pooled relative risk for prevention of relapses of pouchitis for VSL#3 compared to placebo as 0.17 (95%CI: 0.09-0.33). As a result of these conclusions, the multispecies probiotic preparation VSL#3 was granted the A level recommendation for the primary prevention and maintenance of remission of pouchitis after IPAA according to the American Recommendations for probiotic use from 2011^[115]. Furthermore, they suggested that there was some evidence (C level) supporting its use even for the therapy of active pouchitis.

Finally, clinical guidelines for the management of pouchitis from 2009 suggest the use of VSL#3 in patients with recurrence of pouchitis following antibiotic treatment or having several recurrences despite antibiotic therapy^[133]. However, they do not suggest probiotics for the treatment of acute pouchitis.

TREATMENT OF ACTIVE CROHN'S DISEASE

Clinical studies investigating the treatment of active Crohn's disease with probiotics were scarce. Gupta *et al*^[134] reported a very small open-label pilot study of four children with mildly to moderately active Crohn's disease who were treated with entero-coated tablets containing *Lactobacillus rhamnosus* GG (10^{10} CFU) twice daily for 6 mo. Clinical activity was monitored by pediatric Crohn's disease activity index (PCDAI) and changes in intestinal permeability were measured by a double sugar permeability test. A significant improvement in clinical activity was observed 1 wk after starting *Lactobacillus* GG. Median PCDAI scores at 4 wk were 73% lower than baseline. Intestinal permeability improved in an almost parallel fashion. The authors concluded that the findings of this pilot study showed that *Lactobacillus* GG might improve clinical status and gut barrier function in children with mildly to moderately active Crohn's disease. Schultz *et*

al^[135] performed a small randomized, placebo-controlled trial to determine the efficacy of *Lactobacillus rhamnosus* GG in the induction or maintenance of medically-induced remission. Eleven patients with moderate to active Crohn's disease were enrolled to receive either *Lactobacillus* GG (2×10^9 CFU/d) or placebo for six months. In all patients, a tapering steroid regimen was applied for the induction of remission, and all received antibiotics the week before probiotic/placebo intervention was initiated. The primary end point was sustained remission; defined as freedom from relapse after 6 mo. Only 5 patients finished the study, with 2 patients in each group in sustained remission. The median time to relapse was 16 \pm 4 wk in the probiotic and 12 \pm 4.3 wk in the placebo group ($P = 0.5$). In contrast with the results of Gupta *et al*^[134], this study did not demonstrate any benefit of *Lactobacillus* GG in inducing or maintaining medically-induced remission in CD.

Although Butterworth *et al*^[136] in the Cochrane Collaboration review concluded that there was insufficient evidence to make any conclusions about the efficacy of probiotics in inducing remission in CD because of a lack of well-designed clinical studies, the two studies using synbiotics that were not included in this review revealed very promising results^[137,138]. Fujimori *et al*^[137] performed an open-label uncontrolled trial using a synbiotic for the therapy of active refractory Crohn's disease. Ten active CD patients who had failed to achieve remission via an initial therapeutic regimen of aminosacilates and prednisolone were given synbiotic therapy consisting of two probiotic preparations that both contained *Bifidobacterium breve* 3×10^{10} CFU, *Lactobacillus casei* 3×10^{10} CFU, and *Bifidobacterium longum* 1.5×10^{10} CFU, as well as a prebiotic comprised of 3.3-9.9 g of psyllium (*Plantago ovata*). Patients were free to adjust their intake of probiotics or prebiotics throughout the trial. For the assessment of disease activity, Crohn's disease activity index (CDAI), International Organization for the Study of Inflammatory Bowel Disease (IOIBD) score, and blood sample variables were evaluated and compared before and after the trial. The duration of the trial was 13.0 \pm 4.5 mo. Of the ten included patients, 6 had a complete response, one had a partial response, and three were non-responders. Two patients discontinued treatment and four decreased their corticosteroid therapy. Both CDAI and IOIBD scores were significantly reduced after therapy (255-136, $P = 0.009$; 3.5-2.1, $P = 0.03$, respectively), however the laboratory markers of inflammation did not change. With the exception of some abdominal bloating disappearing with discontinuation of psyllium ingestion, there were no adverse events. The authors concluded that a combination of high-dose probiotics and prebiotics could be safely and effectively used as a co-therapy for the treatment of active CD.

Recently, Steed *et al*^[138] conducted a randomized, double-blind, placebo-controlled trial including 35 patients with active CD using a synbiotic therapy comprised of probiotic *Bifidobacterium longum* 4×10^{11} CFU and prebiot-

ic Synergy 1 (oligofructose and inulin) 12g daily. Patients were requested to continue on stable doses of conventional medication they were receiving at initiation of the trial. The patients' clinical status was scored by CDAI and endoscopies with biopsies were performed at the start, and at 3 and 6 mo of therapeutic intervention. Six patients from the synbiotic group and 5 from the placebo group were lost from follow-up. Upon comparing pre- and post-treatment CDAI, there was a significant clinical improvement in the synbiotic group (start 219 ± 74.6 , finish 147 ± 74 ; $P = 0.020$) but not in the placebo group (start 249 ± 79.4 , finish 233 ± 155 ; $P = 0.81$). Similarly, there was a significant improvement in mean histological scores in the synbiotic group (start 6 ± 5 , finish 3 ± 4 ; $P = 0.018$) but not in the placebo group (start 6 ± 5 , finish 5 ± 6 ; $P = 0.75$). A significant reduction of pro-inflammatory TNF- α and an increase of mucosal *Bifidobacteria* was also observed in the synbiotic group.

MAINTENANCE OF REMISSION IN CROHN'S DISEASE

Only a few high-quality studies have been performed to assess the efficacy of probiotics for the maintenance of remission achieved with standard medical therapy or surgical resection in Crohn's disease. Currently, the use of probiotics for the maintenance of remission in Crohn's disease is not recommended.

In a trial by Guslandi *et al.*^[139], 32 patients with Crohn's disease in clinical remission (CDAI < 150) were randomized to treatment with either mesalamine 1 g three times daily or mesalamine 1 g two times daily plus probiotic yeast *Saccharomyces boulardii* 1 g daily for six months. Clinical relapses were observed in 37.5% of patients receiving mesalamine alone but in only 6.25% of patients combining mesalamine with the probiotic ($P = 0.04$). The authors hence concluded that *Saccharomyces boulardii* might be useful in the maintenance treatment of Crohn's disease.

Bousvaros *et al.*^[140] conducted a randomized, placebo-controlled trial of the probiotic *Lactobacillus rhamnosus* GG (LGG) to see whether the addition of LGG to standard therapy prolonged remission in children with CD. Seventy-five children and adolescents from 5 to 21 years old with CD in remission were randomized to receive either LGG ($n = 39$) or placebo ($n = 36$), and followed for up to 2 years. Concomitant medications, including aminosalicylates, 6-mercaptopurine, azathioprine, and low-dose alternate day corticosteroids were allowed. The percentage of patients that relapsed did not significantly differ between the LGG and the placebo group (31% *vs* 17%; $P = 0.18$), neither did the median time to relapse (9.8 mo *vs* 11.0 mo; $P = 0.24$). In conclusion, LGG did not prove to be effective for maintaining remission in children with CD when given as an adjunct to standard therapy. The ineffectiveness of probiotic strain *Lactobacillus rhamnosus* GG for maintenance therapy in CD was also supported by a study by Prantera *et al.*^[141], who performed a randomized placebo-controlled study in patients operated for

Crohn's disease in whom all of the diseased gut had been removed. The patients received 1.2×10^9 CFU of *Lactobacillus* GG or placebo for one year. Ileocolonoscopy was performed at the end of the trial or at the onset of symptoms. Clinical recurrence was ascertained in 16.6% in the LGG group and in 10.5% in the placebo group. Sixty percent of patients in clinical remission on LGG had endoscopic recurrence compared with 35.3% on placebo ($P = 0.297$). There were no significant differences in the severity of the lesions between the two groups. Marteau *et al.*^[142] studied the potential of *Lactobacillus johnsonii* LA1 for prevention of recurrence in operated CD patients. This was a randomized, double-blind, placebo-controlled study. Patients were randomized to receive *Lactobacillus johnsonii* LA1 4×10^9 CFU/d ($n = 48$) or placebo ($n = 50$) for six months. No other treatment was allowed. There were 4 clinical recurrences in the probiotic group and 3 in the placebo group. In patients with symptomatic remission, endoscopic recurrence was observed in 64% in the placebo group compared to 49% in the probiotic group ($P = 0.15$). Endoscopic score distribution did not differ significantly between the two groups. A similar double-blind placebo-controlled study was performed by Van Gossum *et al.*^[143], who randomized 70 patients who had undergone elective ileocecal resection for CD to daily treatment with either *Lactobacillus johnsonii* LA1 10^{10} CFU ($n = 34$) or placebo ($n = 36$) for 12 wk. The primary objective of this study was to assess the effect of probiotics on the endoscopic recurrence rate at 12 wk. Clinical relapse rate was 15% in the probiotic group and 13.5% in the placebo group ($P = 0.79$). The mean endoscopic score at 3 mo was not significantly different between the two groups ($P = 0.48$), nor was the percentage of patients with severe endoscopic recurrence ($P = 0.33$). According to the results of these studies, it seems that, like LGG, *Lactobacillus johnsonii* LA1 has no effect on remission in CD.

With the intention of preventing postoperative recurrence of CD, another two double-blind placebo-controlled trials were performed. Chermesh *et al.*^[144] investigated the use of a synbiotic cocktail of 4 probiotics and 4 prebiotics (Synbiotic 2000), and Madsen *et al.*^[145] used multispecies probiotic VSL#3, which proved to be efficient in the therapy of ulcerative colitis and pouchitis.

In the 2006 Cochrane Collaboration review regarding probiotics for maintenance of remission in CD by Rolfe *et al.*^[146], the authors identified 7 eligible studies. They found no statistically significant benefits of *E. coli* Nissle for reducing the risk of relapse compared to placebo, or for *Lactobacillus rhamnosus* GG after surgical or medically-induced remission. There was no statistically significant benefit of probiotics for reducing the risk of relapse compared to medical maintenance therapy employing aminosalicylates or azathioprine. Moreover, they found more adverse events in *Lactobacillus* GG treated patients. However, a small study using *Saccharomyces boulardii* demonstrated a difference in favor of its use combined with medical maintenance therapy in comparison with standard medical therapy alone, although the difference was

not statistically significant. They concluded that there is no evidence to suggest the use of probiotics for the maintenance of remission in CD. In the second Cochrane Collaboration review analyzing different interventions for the prevention of post-operative recurrence of Crohn's disease, the authors came to the same conclusion that probiotics were not superior to placebo^[147].

Similarly, a meta-analysis performed by Rahimi *et al.*^[148] also failed to demonstrate the efficacy of probiotics in maintaining remission and preventing clinical and endoscopic recurrence in CD. Moreover, in a meta-analysis performed by Shen *et al.*^[149], researchers came to the conclusion that not only were *Lactobacilli* inefficacious, but also that administration of *Lactobacillus* GG might increase the relapse rate.

PREBIOTICS AND IBD

Compared to probiotics, there is considerably less clinical evidence regarding the use of prebiotics for IBD therapy.

In an early trial by Hallert *et al.*^[150], the ingestion efficiency of *Psyllium* (*Plantago ovata*, ispaghula husk) for 4 mo compared to placebo was studied for relieving gastrointestinal symptoms in patients with UC in remission. Regarding the symptom's score, ispaghula was consistently superior to placebo ($P < 0.001$) and was associated with a significantly higher rate of improvement (69% *vs* 24%; $P < 0.001$). Based on these results, the authors suggested that ispaghula could be helpful in the management of gastrointestinal symptoms in UC.

A Spanish group performed a multicenter open-label, randomized clinical trial to assess the efficacy and safety of *Plantago ovata* seeds as compared with mesalamine in maintaining remission in UC^[151]. A total of 105 patients with UC in remission were randomized into three groups treated with *Plantago ovata* (10 g twice daily), mesalamine (500 mg twice daily), or *Plantago ovata* plus mesalamine at the same doses for 12 mo. Three patients, all from the *Plantago ovata* group, were withdrawn because of adverse events (i.e., constipation and/or flatulence). After 12 mo, the treatment failure rate was 40% in the *Plantago ovata* group, 35% in the mesalamine group, and 30% in the *Plantago ovata* plus mesalamine group. The probability of continued remission was similar ($P = 0.67$). A significant increase in fecal butyrate levels was observed in the groups using *Plantago ovata* ($P = 0.018$). The authors concluded that *Plantago ovata* seeds might be as effective as mesalamine for maintenance therapy in UC patients in remission. Furthermore, Casellas *et al.*^[152] conducted a prospective, randomized, placebo-controlled pilot trial comparing the effect of oligofructose-enriched inulin 12 g/d ($n = 10$) and maltodextrin used as placebo ($n = 9$) for 2 wk in patients with mild to moderately active UC. Concomitant treatment with mesalazine (3 g/d) was allowed. A significant reduction of fecal calprotectin, a marker of intestinal inflammation, was observed in the group receiving oligofructose-enriched inulin (day 0: 4377 \pm 659 μ g/g; day 7: 1033 \pm 393 μ g/g, $P < 0.05$) but not

in the placebo group (day 0: 5834 \pm 1563 μ g/g; day 7: 4084 \pm 1395 μ g/g, n.s.).

Hafer *et al.*^[153] investigated the clinical and histological efficacy of lactulose in patients with both UC and CD. In a pilot study, 14 UC and 17 CD patients, most of whom were in a clinically active state, were randomized either to receive 10 g lactulose daily or placebo, adjuvant to standard therapy for 4 mo. No significant improvement of clinical activity index, endoscopic score, or immunohistochemical parameters was observed in CD or UC patients receiving lactulose in comparison to the control group.

Several clinical trials were performed in Japan using germinated barley foodstuff (GBF), which mainly consists of dietary fiber and glutamine-rich protein, for the therapy of UC. Kanauchi *et al.*^[154] investigated the efficacy of long-term administration of GBF in the treatment of active UC in a multi-center open trial. Twenty-one patients with mild to moderate UC received 20-30 g of GBF while baseline treatment with 5-aminosalicylates and/or steroids was continued. After 24 wk of treatment, the GBF group showed a significant decrease in clinical activity index compared with the control group ($P < 0.05$). No side effects related to GBF were observed. The same group published results of another study in which GBS was used for maintenance therapy in UC^[155]. Patients were randomized into two groups: GBF 20 mg/d ($n = 22$) and control ($n = 37$). Response to treatment was assessed by monitoring the clinical activity index (CAI) and endoscopic score. Significantly better CAI values and a significantly lower recurrence rate were observed in the GBF group at 3, 6, and 12 mo compared with the controls. No side effects related to GBF were observed. According to the results of both studies, GBF could reduce the clinical activity of active UC, and appeared to be effective as a maintenance therapy in patients with UC.

Moreover, a small open-label trial was performed by Lindsay *et al.*^[156] in which they treated 10 patients with active ileocolonic Crohn's disease with 15 g of FOS for three weeks. FOS induced a significant reduction in the disease activity index from 9.8 ± 3.1 to 6.9 ± 3.4 ($P < 0.01$). They also observed a significant increase in fecal *Bifidobacteria* concentration, in the percentage of IL-10 positive, and TLR2 and TLR4 expressing dendritic cells in mucosal biopsies.

In contrast to previous findings, the results from a randomized double-blind placebo-controlled trial performed by Benjamin *et al.*^[157] did not confirm the efficacy of FOS for therapy of active CD. In this study patients were randomized to 15 g/d FOS ($n = 54$) or placebo ($n = 49$) for 4 wk. More patients receiving FOS (26% *vs* 8%; $P = 0.018$) withdrew before the 4-wk end point and there was no significant difference in the number of patients achieving a clinical response between the FOS and placebo groups (22% *vs* 39%; $P = 0.067$).

Considering all the above facts regarding the use of prebiotics, there is very little evidence to support their use in IBD therapy. However, supplementation with germinated barley foodstuff, *Psyllium* (*Plantago ovata*, ispaghula

husk), or oligofructose-enriched inulin might provide some benefit in patients with active UC or UC in remission, but more high-quality clinical studies are needed to confirm their effectiveness.

CONCLUSION

Probiotics and prebiotics definitely have great potential for future therapeutic approaches in inflammatory bowel disease. However, further research is required to identify specific probiotic strains or their combinations and prebiotic substances that will be most efficient for therapies of different types and stages of activity of intestinal inflammation.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Clinical characteristics and treatment of inflammatory bowel disease: A comparison of Eastern and Western perspectives

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Abstract

Inflammatory bowel disease (IBD) is a chronic, relapsing intestinal inflammatory disorder with unidentified causes. Both environmental factors and genetic aspects are believed to be crucial to the pathogenesis of IBD. The incidence and prevalence of IBD have recently been increasing throughout Asia, presumably secondary to environmental changes. This increasing trend in IBD epidemiology necessitates specific health care planning and education in Asia. To this end, we must gain a precise understanding of the distinctive clinical and therapeutic characteristics of Asian patients with IBD. The phenotypes of IBD reportedly differ considerably between Asians and Caucasians. Thus, use of the same management strategies for these different populations may not be appropriate. Moreover, investigation of the Asian-specific clinical aspects of IBD offers the possibility of identifying causative factors in the pathogenesis of IBD in this geographical area. Accordingly, this review summarizes current knowledge of the phenotypic manifestations and management practices of patients with IBD, with a special focus on a comparison

of Eastern and Western perspectives.

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Key words: Crohn's disease; Ulcerative colitis; Inflammatory bowel disease; Clinical characteristics; Treatment; Asia

Core tip: Over the past two decades, the incidence and prevalence of inflammatory bowel disease (IBD) have changed with a trend toward increasing across Asia, especially East Asia. This increasing trend in IBD epidemiology necessitates specific health care planning and education in Asia. To this end, we must gain a precise understanding of the distinctive clinical and therapeutic characteristics of Asian patients with IBD, compared to Caucasians patients with IBD. Accordingly, this review summarizes current knowledge of the phenotypic manifestations and management practices of patients with IBD, with a special focus on a comparison of Eastern and Western perspectives.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, idiopathic inflammatory disorder of the gastrointestinal tract without identifiable causes. It comprises primarily Crohn's disease (CD) and ulcerative colitis (UC). IBD was traditionally regarded as being prevalent in mainly Western countries. Over the past two decades, however, the incidence pat-

Table 1 Clinical characteristics of inflammatory bowel disease in Asian and Western countries

Differences	Asian characteristics
Peak age at disease onset	Smaller second peak
Sex distribution of CD	Male predominance
Cigarette smoking in CD	Lower prevalence
CD distribution and behavior	Ileocolonic predominance Similar behavior
UC distribution and behavior	Similar distribution Milder disease course
Familial aggregation in IBD	Lower prevalence
Extraintestinal disease	Lower prevalence
Medical treatments	Lower use of thiopurine and anti-TNF therapy
Surgical treatments	Comparable in CD Lower in UC
UC-associated CRC	Comparable cumulative risk

CD: Crohn's disease; UC: Ulcerative colitis; anti-TNF therapy: anti-Tumor necrosis factor therapy; CRC: Colorectal cancer.

tern of IBD has changed. The incidence in the West has remained relatively stable, while that in Asia has increased markedly^[1]. This recent change in the incidence and prevalence of IBD is attributed to environmental changes.

The growing incidence of IBD in Asia has important implications for those who formulate health care policy plans; these individuals should provide specific health care planning, services, and education while balancing the health needs and social burdens of a given country. However, it also has important implications for clinicians and researchers. According to the published literature, the IBD phenotypes differ considerably between Asians and Caucasians. Nevertheless, most strategies for the prevention and treatment of IBD flare-up in Asia have followed Western guidelines. Gradual accumulation of data on IBD in Asians will facilitate the formulation of Asian-specific practice guidelines; however, direct comparisons of the clinical characteristics of IBD in Asian and Western countries are few. Formulation of appropriate strategies for Asian patients with IBD must begin with an accurate understanding of the different clinical characteristics of the two populations. These include clinical manifestations and therapeutic aspects, as well as epidemiology. To this end, this review summarizes and compares current knowledge of the phenotypic manifestations and management of patients with IBD in Eastern and Western countries. Furthermore, investigation of the Asian-specific clinical aspects of IBD offers the possibility of identifying causative factors in the pathogenesis of IBD, which will also be covered in this review.

DIFFERENCES IN CD BETWEEN THE EAST AND WEST

Clinical characteristics of CD

Peak age of disease onset: Early studies from Western countries reported that CD was characteristically associated with a bimodal age distribution pattern, peaking at

the ages of 20 to 39 years and showing a second smaller peak at the ages of 50 to 79 years^[2-4]. Various peak ages of CD onset have been reported in Asia. A Korean study reported a smaller second peak in the incidence of CD^[5]. There was also a trend toward a second peak in the Hong Kong population^[6]. A recent prospective, population-based study from the Asia-Pacific region also indicated a smaller second peak in the incidence of CD, albeit at a younger age (40-44 years) than that in Western countries^[7]. However, this bimodal presentation has not been uniformly identified in other Asian studies^[8,9]. The reason for the bimodal distribution remains unclear, but some environmental factors are known to be associated with this phenomenon. One hypothesis is that it may be due to certain age-specific environmental factors, such as passive smoking in childhood and active smoking in adulthood. It may also be caused by the different sensitivities of different age groups to certain infectious factors^[10]. We speculate that Asian people with genetic susceptibilities could develop CD at the early peak ages of 20-39 years; however, the later second peak might be smaller than that in the West because environmental factors are less prevalent in Asia. Accordingly, it is expected that the second peak in the incidence of CD in Asia will increase as the region becomes increasingly westernized over time (Table 1).

Male predominance in Asians

European and North American studies have consistently revealed that the incidence of CD in females is equal to or greater than that in males^[2,4,11,12]. In striking contrast, a male predominance in Asian patients with CD has been reported. In all recent studies from Korea, China, and Japan, the male-to-female ratios ranged from 1.67:1 to 2.9:1^[9,13,14]. A recent prospective, population-based study from the Asia-Pacific region also demonstrated a male predominance^[7]. Interestingly, a recent European study indicated a slight male predominance (60%) in adult Eastern European patients with CD^[15]. Smoking, vaccination, or other factors (*e.g.*, geographic, ethnic, and social) might account for this sex difference^[10]. Some Western researchers have suggested that the smoking rates, exposure of social life, or Westernized lifestyles were more prevalent in males than in females in Asia and that males might have more opportunities to receive medical services, including endoscopy, than females in Asia. However, if male predominance is in fact present in Asia, it is possible that changes in susceptible genes in sex chromosomes or changes in sex hormones might be involved in the pathogenesis of CD.

Cigarette smoking

Smoking represents one of the most consistently observed environmental risk factors for CD. Studies in Western countries have shown that smoking is a strong risk factor for the development of CD, but that it protects against the development of UC^[16-20].

Based on recent prospective, population-based cohort

studies, the proportion of current or ex-smokers among Asian patients with CD (11.8%-28.0%)^[7,21] is substantially lower than that among Western (57%) and Eastern European patients (62%)^[15]. A study comparing patients between Melbourne and Hong Kong also reported that fewer patients were current or ex-smokers in Hong Kong (8%) than in Melbourne (50%)^[22].

Western patients with CD who smoke have a worse disease course and are more likely to relapse after medically and surgically induced remission^[23-25]. In terms of flare-up rates and therapeutic needs, disease severity is similar in patients who have never smoked and those who have stopped smoking, and both have a better course than continuing smokers^[23]. Progression to stricturing or penetrating disease and subsequent surgery rates are reduced by smoking cessation^[26]. However, smoking may not have the same effect in CD in different ethnic groups or geographic regions as in Western populations. For example, studies among Israeli Jews showed that smoking is not associated with the risk of CD^[27-29]. More studies on Asian patients with CD are warranted to determine the impact of smoking on the development and progression of disease and its association with the disease phenotype in this population. Our hypothesis is that the lower rate of cigarette smoking among Asians may be one of the factors related to their better prognosis, as indicated by the lower rate of surgery in Asian than in Western patients with CD.

Familial aggregation

Previous studies have suggested that patients with IBD have less family clustering in East Asia. Studies from Asia have collectively reported that familial aggregation rates range from 0.0% to 3.0%^[6,30-35], which is clearly lower than that in the Western population (13.4%)^[36]. However, familial aggregation rates appear to be higher in West Asia, ranging from 12.9% to 19.0%^[1,37]. Recent studies from Korea have suggested that the familial aggregation rate of IBD may increase with time, in parallel with the increase in the prevalence of IBD within the country^[15,31]. Data on the familial clustering rates in other Asian countries must be validated by a longitudinal prospective cohort study. Moreover, it should be tested whether patients with family history of IBD would have a worse disease outcome than those without. Our own study showed no difference in clinical characteristics and outcomes between them^[38]. We speculate that both environmental and genetic factors contribute to the low familial aggregation rates among Asian patients with CD. It might be valuable to elucidate the Asian-specific pathogenesis and establish prevention methods by investigating Asia-specific lifestyles or environmental factors, including breast feeding, tonsillectomy, childhood vaccination, infectious disease, or dietary intake of fiber and sugar.

Disease location

In the West, CD has been found to occur in the ileum, colon, and both the ileum and colon in equal proportions

of patients^[15,39]. However, a number of studies from the West have reported isolated colonic disease to be the most common type of CD^[11,40,41]. A study comparing patients in China and the US also reported that American white patients have more colorectal involvement^[32]. However, ileocolonic disease appears to be the most common type of CD in Asia^[5,7,21,33,34,42,43]. CD confined to the small bowel is also common in Asia. Western guidelines suggest a follow-up colonoscopy to assess mucosal healing or recurrence after biologic therapy or surgery. However, routine ileocolonoscopy for therapeutic monitoring or after surgery might be less useful in Asian patients. Instead, radiological evaluation might play a role in such cases because isolated ileal or ileocolonic disease is not entirely visible by ileocolonoscopy.

Upper gastrointestinal tract involvement, which is significantly associated with disease prognosis such as changes in behavior^[44] or the need for early surgery or hospitalization^[45], has been rarely reported in Asia. This might be due to the lack of consensus regarding the performance of routine gastroduodenoscopy in patients with newly diagnosed CD in real practice. A study from China found that 23.5% of patients had upper gastrointestinal tract involvement at the time of diagnosis^[14], but other studies^[3,7,11,46,47] from both the Asia-Pacific region and the West have reported lower proportions. In a recent Korean study, jejunal involvement was observed in 14.1% of patients with CD at the time of diagnosis^[48]. This markedly different result compared to China might be an overestimation. Thus, further larger studies are needed to determine the proportion of upper gastrointestinal involvement in patients with CD in Asia.

Disease behavior and course

Studies from the West have shown that the rates of inflammation, stricture, penetration, and perianal fistulas in CD at the time of diagnosis are 62%-81%, 5%-27%, 8%-14%, and 10%-27%, respectively^[11,44,47,49,50]. Similarly, Asian studies have reported inflammatory disease in 40% to 69%, stricturing disease in 20%-29%, and penetrating disease in 10%-31% of patients with CD^[13,21,33,34,45,51,52]. Interestingly, several studies documenting the disease behavior at the time of diagnosis have reported higher proportions of perianal fistula at the time of diagnosis in Hong Kong (33.3%)^[33], Korea (36.7%)^[34], and China (58.8%)^[14] than in the West. Perianal fistula is known to be a poor prognostic factor in patients with CD^[44,47]. In general, however, the prognosis of Asian patients with CD patients is reportedly better than that of Western patients with CD. Whether the presence of perianal fistula is actually a poor prognostic factor of CD remains to be determined and warrants further study. Actually, perianal fistula is described as independent from the penetrating type in the Montreal classification.

The evolution from inflammatory behavior to a more complicated disease behavior (stricturing or penetrating) is well demonstrated in the Western literature^[46,47,53], and has also been shown in Asian studies from Hong Kong^[33]

and Korea^[54].

Based on long-term follow-up studies from Japan, it appears that Japanese patients with CD have a long-term prognosis similar to that of their Western counterparts regarding cumulative operation rates^[43]. However, compared with Western patients with CD^[55], a recent study from Korea showed a better prognosis with respect to the incidence of surgery^[13,34]. This can be partly explained by the conservative attitude regarding bowel surgery among Korean physicians and patients or the smaller numbers of patients with a severe phenotype because of the short history of CD in the Asian region, including Korea.

Extraintestinal manifestations

Extraintestinal manifestations (EIM) have been reported in Asian patients with CD with widely variable rates: 19.0%-58.8% in China^[14,52,56] and 25.0% in Hong Kong^[6]. Among EIM, a high rate of ankylosing spondylitis (9%) among patients with CD has been reported^[6]. A study comparing patients between the US and China reported that American white patients developed more EIM (40% *vs* 20%; OR = 2.63; *P* = 0.013)^[32], especially chronic arthralgia (32% *vs* 4%; OR = 13.07; *P* < 0.001). However, it is difficult to directly compare the prevalence of EIM between Asian and Western patients with CD because most Asian reports were hospital-based (not population-based) retrospective studies or prospective cohort studies involving small numbers of patients (*n* = 17, 58.8%)^[14]. Moreover, they were performed under different diagnostic criteria for EIM. Generally, the prevalence of EIM in Asia is accepted to be similar or slightly lower than that in the West (19%-25% *vs* 21%-41%, respectively)^[57-60]. Less frequent EIM may be associated with the relatively better prognosis among Asian patients than in Western patients because EIM, especially ankylosing spondylitis, is known to be associated with a poorer prognosis in patients with IBD.

TREATMENT OF CD: COMPARISON BETWEEN THE EAST AND WEST

Medical treatments

Conventional therapy: The use of corticosteroids for CD is variable in studies from Asia. In one study, Asian specialists used corticosteroids as the first-line treatment in 50% of patients with mild CD and 84% of patients with moderate CD^[61]. Moreover, they used corticosteroids as a maintenance therapy in approximately 25% of patients with CD^[61], which might appear to be inappropriately high because corticosteroids have more side effects than placebo or low-dose 5-aminosalicylic acids (5-ASA)^[62]. In a recent prospective European study, 54%-55% of patients with CD received corticosteroid therapy as an initial treatment during the first 3 mo of disease in Eastern and Western European centers, but did not during the maintenance phase^[15].

Among patients with CD, the overall response rates to corticosteroids in Asia were similar to or better than

those in the West. The short-term response rates at 1 mo were > 80% in both Asia and the West (Figure 1A), and 56.6% and 32.0% of patients, respectively, were corticosteroid-free without the need for surgery at 1 year (Figure 1B)^[63,64].

The use of thiopurines in Asia also varies among countries. A Korean single-center study reported that thiopurines were used in 63% of patients with CD^[34]. A separate Korean study showed that 42% of patients received thiopurines and that the cumulative thiopurine requirement was 9.1% at 1 year, 32.2% at 5 years, and 51.6% at 10 years^[65]. Another cross-sectional study from Hong Kong noted that thiopurines were used in 63.6% of patients^[22]. The authors of this study stated that the use of thiopurines was significantly less frequent in Hong Kong patients than in Melbourne patients (63.6% *vs* 82.1%, respectively, *P* < 0.001). A single-center review from East China found that 61 of 227 (26.9%) patients had indications for immunomodulator use. However, such agents were prescribed to only 34.0% of the patients, and of these 34.0%, 38.0% received a subtherapeutic dose with no attempt to increase the dose^[66]. A recent prospective, population-based study from the Asia-Pacific region reported that only 35% of patients with CD received immunomodulator therapy^[7]. Taken together, these results indicate that Asian physicians have a tendency to inappropriately use large amounts of corticosteroids and insufficient amounts of thiopurines. It is important to educate physicians regarding these medications.

There appears to be a higher rate of adverse events, particularly leukopenia, in Asians than in Caucasians when taking thiopurines. Up to 40%-56% of Asian patients may reach the criterion for leukopenia, namely a white blood cell count of < 4000/mm³^[67,68]. The cumulative incidence of myelotoxicity in the Western population was reported to be about 7%^[69]. In Asia, however, thiopurine methyltransferase genotyping itself may not be as helpful in identifying patients who are expected to develop myelotoxicity as in Western countries^[70], and the metabolites of thiopurines are not widely measured in real practice in many Asian countries. In Korea, therefore, physicians usually start a thiopurine at a smaller dose (*e.g.*, 25 or 50 mg of azathioprine) and gradually increase the dose with regular evaluations of the white blood cell count instead of determining the metabolite concentration. Alternatively, they may maintain lower doses of thiopurines than recommended in the Western guidelines^[71].

Biologics: In a recent prospective, population-based cohort study from Europe, the rates of biologics use as an initial treatment during the first 3 mo of disease were reportedly 7% and 2% in Western and Eastern European centers, respectively^[15]. This approach was interpreted as top-down therapy or rapid accelerating therapy. However, the economic burden or the reimbursement system can be obstacles to the use of biologics in the very early phase after diagnosis of CD in many Asian countries. An

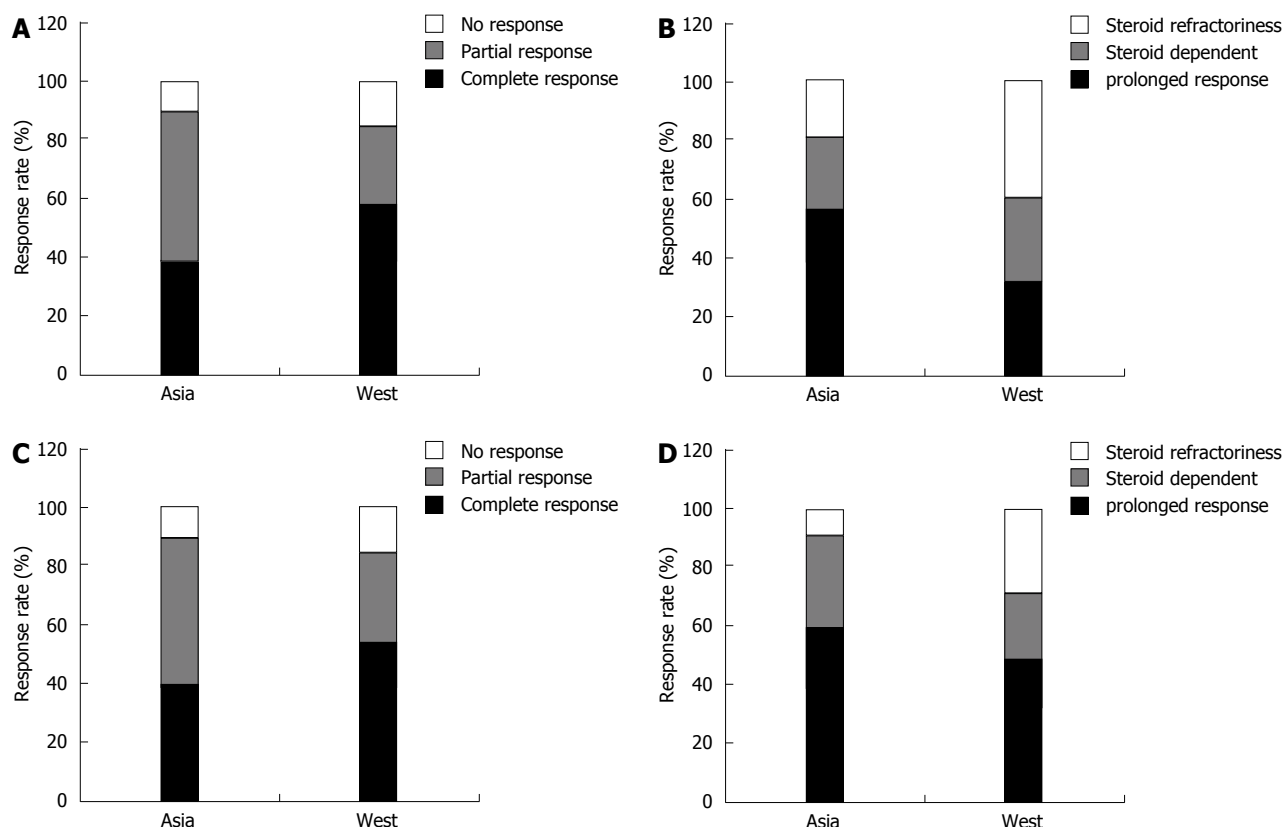


Figure 1 Comparison of the response to corticosteroids between Asia and the West^[63,64,112]. A: Crohn's disease, 1 mo; B: Crohn's disease, 1 year; C: Ulcerative colitis, 1 mo; D: Ulcerative colitis, 1 year.

Asian survey of IBD management practices in different countries found that no IBD specialists would consider anti-tumor necrosis factor (anti-TNF) agents as the first choice for the treatment of CD. Moreover, only 20% considered anti-TNF agents as the second choice^[61]. In a cross-sectional study comparing the management of CD between Melbourne and Hong Kong, a significantly higher number of patients in Melbourne had been on anti-TNF agents than in Hong Kong (40% *vs* 11%)^[22]. A retrospective study from Korea reported that 8.6% of patients with CD used infliximab^[34].

The response rates to infliximab in Asia are similar to or higher than those in the West. Although it is difficult to directly compare the results of responses to infliximab in Asia and the West because of their different designs, the response rates at 2 wk after beginning induction therapy were 72% and 62% in Korea (unpublished retrospective data) and the West^[72], respectively. Moreover, the response rates at 30 and 54 wk after beginning maintenance therapy were 91.7% *vs* 50.0% and 74.7% *vs* 39.0% in Korea (unpublished retrospective data) and the West^[72], respectively. A similar pattern was reported in patients with fistulizing CD between Korea and Western countries. In Asia, anti-TNF agents are used less frequently because of the limited, strict indications under insurance coverage rules, and because of the social economic burden. Moreover, many Asian physicians have not accepted the latest treatment trends, such as rapid accelerated step-up or top-down therapeutic approaches. Again, it is nec-

essary to educate Asian physicians regarding the adequate use of the latest medical treatments for IBD.

Complementary and alternative medicines: There are diverse rates of use of complementary and alternative medicines (CAMs) across Asia. One study from China reported that 90% of patients used concomitant traditional Chinese medications^[73]. Moreover, various proportions of Western patients with IBD use CAM, ranging from 23% to 49% in recent studies^[74-77]. In seven randomized controlled trials of patients with CD, *Artemisia absinthium* (wormwood) and *Tripterygium wilfordii* were superior to placebo in terms of inducing remission and preventing clinical recurrence of postoperative CD, respectively^[78]. In two systematic reviews, omega-3 fatty acids did not appear to be effective for the maintenance of CD remission^[79,80]. Effective anti-inflammatory moieties have yet to be defined.

Another problem is the indiscriminate use of various CAMs without sufficient evidence in patients with IBD. More studies are needed to determine the efficacy and safety of CAM in such patients. In addition, the development of new CAMs from natural products would be helpful in situations in which few drugs are available for patients with IBD.

Surgical treatments

The cumulative operation rates for CD in Japanese studies are comparable to those of Western cohorts, ranging

from 25.9% to 44.4% at 5 years and 46.3% to 80.1% at 10 years, respectively^[43,81]. The surgical resection rate for patients with CD in Hong Kong (29% at 10 years)^[6] is similar to that in a population-based study from Norway, which reported a 10-year cumulative surgical rate of 37.9%^[55]. In a cross-sectional study, there was also no significant difference in the proportion of patients with CD who underwent surgery in Melbourne compared with in Hong Kong (55.1% *vs* 46.0%, respectively, $P = 0.065$)^[22]. Similarly, the cumulative probability of intestinal resection in Korea was reported to be 15.5% after 1 year, 25.0% after 5 years, and 32.8% after 10-15 years. Asian patients with CD are currently considered to have a similar or slightly lower rate of surgery than that among their Western counterparts^[34]. The lack of long-term follow-up studies in Asia makes it difficult to draw a concrete conclusion regarding the cumulative surgical resection rates between Asia and the West.

DIFFERENCES IN UC BETWEEN THE EAST AND WEST

Clinical characteristics of UC

Age and sex: The median age at the time of diagnosis of UC among patients in Asia is similar to or slightly older than that among patients in the West (35-44 years in Asia^[5,9,51,82-85] and 30-40 years in the West^[39,86,87]). In a cross-sectional study, patients with UC in Hong Kong were diagnosed at an older age than Caucasians in Melbourne (median age, 38 years *vs* 30 years, respectively). The authors suggested that this may be partly explained by a delay in diagnosis in Hong Kong^[22]. Another possible explanation would be a weaker influence of genetic factors in Asian patients, which delays disease occurrence (Table 1).

The majority of studies from the West have shown an equal sex distribution for UC, although some reported a male predominance^[86,88]. A growing number of studies in Asia have shown an equal sex distribution^[5,84,85,87,89] or slight male predominance^[9,35,52,82,90]. Collectively, the age and sex distributions of patients with UC are not largely different between the East and West.

Family history: Studies in Asia have reported a family history in 0.0%-3.4% of patients with UC^[35,82,85]. This figure is lower than the 10%-25% reported in Western countries^[37]. A recent population-based cohort study conducted in the Asia-Pacific area showed a family history in 3% of patients in Asia and in 17% of patients in Australia ($P < 0.001$)^[7]. Interestingly, in Korea, an increase in the prevalence of a positive family history from 1.3% in 2001 to 2.7% in 2005^[5] paralleled the increased incidence of IBD. This suggests that the low prevalence of a family history may be a reflection of the low population prevalence and will probably change with time.

In terms of genetic associations, a previous Japanese genome-wide association study (GWAS) and a recent Korean GWAS showed considerable overlap of genetic

associations for UC between Asia and the West. Despite the overlap of genetic associations of Asian and Western patients with UC, as can be seen by the lower proportion of patients with a family history of UC in Asia compared with the West, we realize that UC is one of the principal forms of IBD with complex manifestations, and genetic factors that account for only a portion of the overall disease development. This indicates a need to better explore gene-environment interactions or Asia-specific environmental factors of etiological importance in the development of IBD.

Disease extent: In UC, the extent of disease is classified into three types: proctitis, left-sided colitis, and extensive colitis. In Western population-based studies, these three types comprise 30%-60% of cases, 16%-40% of cases, and 18%-35% of cases, respectively^[91-93]. In Asian population-based studies, they comprise 25.0%-43.7%, 31.0%-31.4%, and 24.9%-39.0%, respectively^[5,42]. Most hospital-based studies in Asia have shown a trend toward a lower proportion of proctitis (8.5%-38.4%), higher proportion of left-sided colitis (29.7%-70.2%), and similar proportion of extensive colitis (21.3%-42.4%) compared with population-based studies^[35,52,84,85,94], indicating that more severe cases were recruited into the hospital-based studies.

In terms of age and disease extent, several studies have reported that the extent of UC at diagnosis differs significantly according to age at diagnosis^[83,95]. In one Korean study, proctitis was more common in elderly patients (28.9% in the young group *vs* 33.8% in the elderly group) and extensive colitis was more common in younger patients (35.1% in the young group *vs* 22.5% in the elderly group), suggesting a poorer clinical outcome in younger patients ($P < 0.05$)^[83].

In a recent comparative epidemiological study of IBD across Asia and the Pacific, the extent of UC was classified as proctitis in 37%, left-sided colitis in 32%, and extensive colitis in 31%. These results are not significantly different from those in a study from Australia, which classified UC as proctitis in 32%, left-sided colitis in 27%, and extensive colitis in 41% (all $P > 0.05$)^[7]. A recent population-based cohort study from the West and East Europe showed that the ratios of disease extent for UC from Western and Eastern European centers were proctitis in 20% and 22%, left-sided colitis in 41% and 46%, and extensive colitis in 38% and 32%, respectively^[15]. Collectively, a slightly higher proportion of extensive colitis is observed in Western countries than in Asia, suggesting a more favorable prognosis of UC in Asia.

Disease course: Although definitions of clinical relapse and measurements of disease severity vary among studies, most suggest that Asian patients with UC have a milder disease course than do patients from Western countries^[82,90,96]. In Asian studies, most patients with UC had a chronic relapsing disease course rather than continuous active disease^[84,97], similar to Western data^[98]. In a Malay-

sian study, the rate of maintaining remission was reported as 64.3% of patients with UC at 10 years after diagnosis, the rate of a chronic relapsing disease course as 25%, and the rate of chronic persistent disease with low or high activity as 3% after 10 years^[97]. In a Norwegian study, the rate of maintaining remission was reported to be 55% of patients with UC at 10 years after diagnosis, which are slightly lower than the Malaysian data^[98], and the rate of a chronic relapsing disease course to be 37%. In a Korean study, the cumulative relapse rate at 10 years after diagnosis (88.4%) was similar to that of a Western study (83%). However, the cumulative probabilities of colectomy (2.0% after 1 year, 2.8% after 3 years, and 3.3% after 5-15 years)^[96] were lower than those in a Western study (3.5% after 1 year, 7.6% after 5 years, and 9.8% after 10 years)^[98].

Based on the larger numbers of patients with a remission status, lower numbers of patients with a chronic relapsing or persistent active disease course, and lower cumulative operation rates, Asian patients with UC appear to have a milder disease course than that of Western patients with UC. However, the above-mentioned lower cumulative surgical rates may also be associated with diversity in management strategies or different levels of acceptance of colectomy by physicians and/or patients between Asia and the West in addition to disease severity.

Incidence of colorectal cancer

The overall prevalence of colorectal cancer (CRC) associated with UC is reportedly 3%-5% in the West^[99] and 0.0% to 2.2% in Asia^[35,52,85,90,96,100-103]. In a previous meta-analysis from the West, the cumulative risk of CRC associated with UC was reportedly 1.6%, 8.3%, and 18.4% at 10, 20, and 30 years, respectively^[104]. In Asian studies, the cumulative risk of CRC in patients with UC was reportedly 0.70% to 1.15% at 10 years, 3.56%-7.90% at 20 years, and 14.4%-33.2% at 30 years^[100,103], which is comparable with Western cohorts. However, considering the hospital-based design of most Asian studies, the actual corresponding risks might be lower than estimated.

Recently, however, a Western report showed that the risk of CRC decreased from 1979 to 2008 (RR = 1.34 in 1979-1988 to 0.57 in 1999-2008) and that the overall risk of CRC among patients with UC was comparable with that of the general population (RR = 1.07; 95%CI: 0.95-1.21). These findings suggest that a diagnosis of UC no longer seems to increase patients' risk of CRC. However, subgroups of patients with UC, including those diagnosed with UC in childhood or as adolescents, those with a long duration of disease, and those with concomitant primary sclerosing cholangitis, remain at increased risk^[105].

Current evidence indicates that the risk of CRC in Asian patients with UC is slightly lower than that in Western patients. There is a chance that the prevalence of CRC will increase with the rising incidence and increasing proportion of patients with a longer follow-up in Asian countries. A very recent single-center study in Korea showed a substantial increase in CRC among patients with long-standing UC, which is comparable to Western data (unpublished data). Long-term prospective follow-

up studies are warranted to estimate the actual risk of CRC in Asian patients with UC.

Extraintestinal manifestations

Although there are limited numbers of long-term prospective cohort studies and variations in the definition of EIM in Asian studies, the prevalence of EIM in UC seems to be lower in Asian than in Western countries^[58,59,73]. The most commonly involved sites of EIM differ between Asia and the West. The most commonly involved site of EIM is the joints in Asian patients with UC (2.0%-19.5%). Next, eye and skin involvement accounts for 0.0%-4.2% and 0.0%-4.2%, respectively^[35,52,82,90,97]. In contrast, eye involvement (iritis/uveitis) in females (3.8%) and primary sclerosing cholangitis (PSC) in males (3.0%) were the most commonly involved sites in a Western population-based study^[58].

PSC associated with UC is less prevalent in Asia (0.0%-1.7%)^[52,84,96,97,106] than in the West (1.6-7.0%)^[59,107]. Because PSC is associated with a risk of CRC in patients with UC, a diagnosis of PSC should not be neglected in real practice despite the low prevalence of PSC in Asia.

TREATMENT OF UC: COMPARISON BETWEEN THE EAST AND WEST

Medical treatments

Conventional therapy: In a recent population-based cohort study in the Asia-Pacific area that compared UC treatments in the first year between Asia and Australia^[7], treatment with antibiotics (22% *vs* 14%, $P = 0.15$) and immunomodulators (thiopurine or methotrexate) (18% *vs* 9%, $P = 0.05$) did not differ between Asia and Australia. Mesalazine (79% *vs* 62%, $P = 0.012$) and corticosteroids (62% *vs* 28%, $P < 0.0001$) were more commonly prescribed for IBD at the time of diagnosis in Australia than in Asia. Topical therapy (mesalazine or corticosteroids) for UC was also more frequently prescribed in Australia than in Asia (55% *vs* 28%, $P = 0.04$).

In a recent population-based European study, 69 (44%) patients with left-sided colitis and 19 (23%) with extensive colitis in Western Europe received more frequently receive combination therapy with oral and topical 5-ASA compared with 21 (42%) and 6 (22%), respectively, in Eastern Europe^[15]. In this study, 26%-33% of patients with UC received corticosteroid therapy as initial treatment during the first 3 mo of disease in Eastern and Western European centers.

In an Asian survey of management practices for IBD in different countries^[61], 83%, 75%, and 61% of respondents preferred 5-ASA, a combination of topical and oral 5-ASA, and corticosteroids, respectively, as induction treatment of mild-to-moderate UC. Almost all respondents agreed that maintenance therapy should be recommended for patients with IBD in remission, with most recommending the use of 5-ASA to maintain remission in UC (91%). However, they also replied that thiopurines and corticosteroids were needed to maintain the remis-

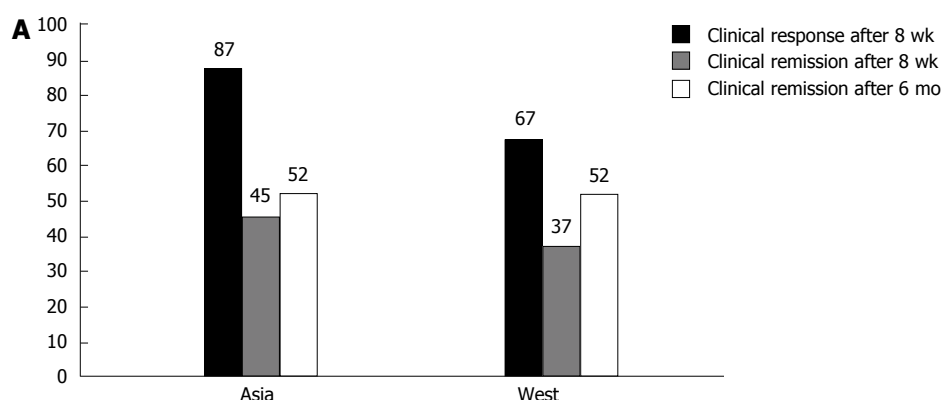


Figure 2 Comparison of the response to infliximab in patients with ulcerative colitis between Asia and the West^[113-115].

sion in approximately 30% and 13% of patients with UC, respectively.

In one cross-sectional study, there was less use of corticosteroids (15.3% *vs* 46.5%, $P < 0.001$) and thiopurines (19.7% *vs* 55.3%, $P < 0.001$) for UC in Hong Kong than in Melbourne, which also reflects differences in practice according to region^[22]. The authors suggested that Asian physicians prefer to manage UC with less intense medical treatments despite more extensive UC and that they use less thiopurines for maintenance therapy compared with the physicians in Melbourne. Again, it is important to educate Asian physicians in terms of following the adequate use of medical treatments according to the practice guidelines for IBD.

Among the UC patients who receive 5-ASA or sulfasalazine therapy, 49.6%^[108] and 72.0% to 75.0%^[109,110] experienced disease relapse in Asia and the West, respectively. The cumulative relapse rate was 21.5% after 1 year, 36.5% after 2 years, 46.9% after 3 years, and 59.8% after 5 years during maintenance therapy with 5-ASA/sulfasalazine, and both the disease extent at diagnosis and anemia were major predictive factors for clinical relapse after 5-ASA/sulfasalazine therapy for Korean patients with mild to moderate UC^[108].

The overall response rates of Asian patients with UC to corticosteroids are similar to or better than those of patients in the West. Short-term response rates at 1 mo were more than 89.2% and 84.0% in Asia and the West (Figure 1C), and 59.4% and 49.0% showed a prolonged response at 1 year, respectively (Figure 1D)^[64,111].

Biologics

A significant difference in the use of anti-TNF inhibitors between Hong Kong and Melbourne has been shown (2/203 *vs* 12/159, $P = 0.001$)^[22]. An Asian survey of IBD management practices in different countries found that < 15% of Asian physicians would use anti-TNF therapy in the management of UC^[61]. In many countries in Asia, the use of biologic agents is self-financed, making the high cost an obstacle to their wider use. However, a short-term population-based study showed that treatment with biological therapy in the first year after diagnosis (2.0% *vs* 0.0%, $P = 0.21$) did not dif-

fer between Asia and Australia^[7]. Long-term follow-up studies are needed to show the chronological trends in the use of anti-TNF therapy in Asia. Emerging studies suggest that anti-TNF therapies are effective and safe in Asian patients with UC. The rates of clinical response and remission to infliximab were 87% and 45% in patients with UC at week 8^[112], which is slightly higher than the rate of clinical response in the West (69.4%-64.5% at week 8)^[113] (Figure 2). Data on long-term efficacy were obtained from 85 of 134 Korean patients who were followed up for more than 6 mo after the first dose of infliximab, and 44 of them (52%) were in remission, which is compatible with UC data in the West^[114]. An another recent Korea study reported that 66.3% of patients demonstrated a clinical response at week 8, 32.6% of whom were determined to be in clinical remission^[115]; this is similar to the response rates in previous Western reports. Collectively, a similar or slightly higher response rate of infliximab in patients with UC is observed in Asia than in Western countries. In the future, it is expected that other anti-TNF agents, such as adalimumab and golimumab, will be used more widely in addition to infliximab in patients with UC in Asian countries.

Complementary and alternative medicines

In 14 randomized controlled trials of patients with UC, aloe vera gel, *Triticum aestivum* (wheat grass juice), *Andrographis paniculata* extract (HMPL-004), and topical Xilei San were superior to placebo in inducing remission or response, and curcumin was superior to placebo in maintaining remission. *Boswellia serrata* gum resin and *Plantago ovata* seeds were as effective as mesalazine, whereas *Oenothera biennis* (evening primrose oil) was not effective and had relapse rates similar to those of omega-3 fatty acids in the treatment of UC^[78]. Larger controlled studies with stricter endpoints and better-defined patient groups are required to obtain more conclusive findings regarding the use of CAM in IBD.

CONCLUSION

Over the past two decades, the incidence and prevalence of IBD have changed with a trend toward increasing

across Asia, especially East Asia. A younger second peak age of disease onset has been shown in Asian populations with CD compared to in Western populations. There is a predominance of male sex and ileocolonic involvement among Asian patients with CD. In patients with UC, the age and sex distribution are not different between Asia and the West. The proportion of current or ex-smokers among Asian patients with CD is lower than that among Western patients. The familial aggregation rates of patients with CD and UC are lower in Asia, but appear to be higher in the West. The disease extent in Asian patients with UC is not significantly different from, and may be slightly less severe than, that in Western populations. Asian patients with UC seem to have a milder disease course than do patients in Western countries. PSC associated with UC is less prevalent in Asia than in the West. Cumulative surgical resection rates in patients with CD do not appear to be different between Asia and the West despite the lack of large-scale, long-term follow-up studies in Asia. Whereas the cumulative surgical resection rates in Asian patients with UC are lower than those in Western patients, the cumulative risk of CRC associated with UC among Asian patients with UC is reportedly comparable with that among Western patients. The use of thiopurine or biologics in patients with IBD remains less frequent in Asia than in the West. There appears to be a higher rate of adverse events, particularly myelotoxicity, in Asians than in Caucasians prescribed thiopurines. The treatment responses for corticosteroids, thiopurines, and biologics of Asian patients with IBD are slightly better than or comparable to those of Western patients.

Several recent prospective, population-based cohort studies were conducted in Asia. Long-term follow-up results from these cohort studies are warranted to help clinicians and researchers further objectively compare the disease prognosis between Asian and Western countries, provide specific health care planning and education, and offer the possibility of identifying causative factors in a population with a rapidly increasing incidence in Asia.

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Impact of *Clostridium difficile* infection on inflammatory bowel disease outcome: A review

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Abstract

Although a considerable number of studies support a substantial increase in incidence, severity, and health-care costs for *Clostridium difficile* infection (CDI) in inflammatory bowel disease (IBD), only few evaluate its impact on IBD outcome. Medline and several other electronic databases from January 1993 to October 2013 were searched in order to identify potentially relevant literature. Most of the studies showed that IBD patients with CDI present a greater proportion of worse outcomes than those without CDI. These patients have longer length of hospital stay, higher rates of colectomies, and increased mortality. Patients with ulcerative colitis are more susceptible to CDI and have more severe outcomes than those with Crohn's disease. However, studies reported variable results in both short-

and long-term outcomes. Contrasting results were also found between studies using nationwide data and those reporting from single-center, or between some North-American and European studies. An important limitation of all studies analyzed was their retrospective design. Due to contrasting data often provided by retrospective studies, further prospective multi-center studies are necessary to evaluate CDI impact on IBD outcome. Until then, a rapid diagnosis and adequate therapy of infection are of paramount importance to improve IBD patients' outcome. The aim of this article is to provide up to date information regarding CDI impact on outcome in IBD patients.

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Key words: *Clostridium difficile* infection; Ulcerative colitis; Crohn's disease; Outcome

Core tip: This review summarizes the impact of *Clostridium difficile* infection (CDI) on inflammatory bowel disease (IBD) outcome. Most of the studies showed that IBD patients with CDI have more of the whole range of short- and long-term worst outcomes than those without CDI. Patients with ulcerative colitis have more severe outcomes than those with Crohn's disease. A prompt diagnosis and adequate treatment of CDI are of paramount importance to improve IBD patients' outcome.

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INTRODUCTION

Over the past 15 years, both incidence and severity of

Clostridium difficile (*C. difficile*) infection (CDI) have increased dramatically worldwide^[1,2]. In addition to broad-spectrum antibiotic therapy^[3,4], other potential risk factors such as advanced age, prolonged hospitalization, immunosuppression, multiple co-morbidities, the use of proton pump inhibitors, and the occurrence of a hypervirulent strain of *C. difficile* known as NAP1 (North American pulsed-field type 1) in some North-American and European areas, have been identified^[5-10].

Referring to the same period, several studies clearly demonstrated a significant increase in CDI incidence in patients with inflammatory bowel diseases (IBD)^[11-17]. Both ulcerative colitis (UC) and Crohn's disease (CD) present high-risk for CDI, although patients with UC are more susceptible than those with CD^[11,12,15,16]. Overall, IBD patients with CDI show more of the whole range of short- and long-term worst outcomes than those without CDI or with CDI alone^[11-13,16-19]. However, studies report variable results concerning mortality and colectomy rates, length of hospital stay, and healthcare costs for IBD patients with CDI^[11,13,14,16,18-21].

This review aims to summarize available literature regarding CDI impact on both short- and long-term outcome in adult IBD patients.

RESEARCH

A systematic literature search was performed on Medline/PubMed, EMBASE, Scopus, Science Direct, CINAHL, and Web of Science (ISI Web of Knowledge) databases from January 1993 to October 2013 using various combinations of the following key words: "inflammatory bowel disease", "ulcerative colitis", "Crohn's disease" and "*Clostridium difficile* infection", "*Clostridium difficile*-associated diarrhea", "pseudomembranous colitis". We included only English written studies carried out on adults, from all geographic regions. A manual search of references from the identified studies was also undertaken to identify any additional studies that may have been missed in the computed-assisted literature search. As our objective was to assess the impact of CDI on IBD patients' outcome, only studies reporting outcome of IBD patients co-infected with CDI were taken into analysis. The following data were extracted from each study included: length of hospital stay, colectomy rate, mortality, and healthcare costs. In addition, given the increased need for surgical intervention in UC patients with CDI, a short review of *Clostridium difficile* enteritis and pouchitis has also been made.

SHORT-TERM OUTCOMES

Length of hospital stay

Studies report different results concerning the length of hospital stay in IBD patients with CDI: some report similar stays^[12,19], some shorter ones^[14], while others (Table 1)^[11,13,16,17,21] refer to longer stays than in patients without CDI or with CDI alone. Jodorkovsky *et al.*^[19] reported

a similar mean length of hospital stay in days for IBD (UC) patients with superimposed CDI and those without CDI (11.7 *vs* 11.0, $P = 0.70$), while Bossuyt *et al.*^[14] found significantly shorter stays in IBD patients with CDI (mean 15.2 d) as compared to non-IBD patients co-infected with *C. difficile* (mean 27.7 d) ($P < 0.001$). By contrast, other studies reported longer length of hospitalization in IBD patients with CDI than in those with IBD alone^[11,13,16,17,21]. Thus, Issa *et al.*^[11], in a retrospective, observational study evaluating IBD patients followed in the Inflammatory Bowel Disease Center, Medical College of Wisconsin, Milwaukee, United States, found a mean length of hospital stay of 13.5 d for their UC patients admitted with CDI as compared to 6 d for those without CDI. From the same center, in a study using nationwide data (including over 2000 IBD patients with CDI, over 44000 with CDI alone, and more than 77000 with IBD alone) was found a 3-d longer hospital stay in IBD patients with CDI. Nguyen *et al.*^[16] using the Nationwide Inpatient Sample (NIS) reported a 65% increase in the number of days for CD and 46% for UC patients with CDI as compared to non-IBD patients. A recent study using Hospital Episodes Statistics (HES) which covers all in-patient activity delivered by NHS hospitals in England, reported a 27.9 d longer hospital stay in patients with IBD complicated by hospital-acquired CDI than in those with IBD alone^[17], much higher than in the above two mentioned North-American studies using similar national datasets^[13,16], the difference being partially accounted for by data collection methods. From Canada, a retrospective population-based cohort study of 181 UC patients with CDI and 1835 without CDI hospitalized in Ontario, between 2002-2008, reported a significantly increased mean length of hospital stay in UC patients co-infected with *C. difficile* (11 d *vs* 6 d, $P = 0.0001$)^[21].

Colectomy rate

Contrasting results have also been reported regarding colectomy rate in IBD patients with superimposed CDI. Analysis of the NIS HCUP (Healthcare Cost and Utilization Project) data containing more than 90% of United States community hospital discharges^[22], showed a six fold (OR = 6.6; 95%CI: 4.7-9.3) increase in colectomy rate in IBD patients with concomitant CDI in comparison with CDI patients without underlying IBD^[13]. It should be underlined that this analysis also includes IBD patients admitted for elective surgery, a fact which contributed to such a high colectomy rate^[23]. However, other studies too reported higher colectomy rates in IBD patients with CDI than in CDI-free IBD population or in patients with CDI alone^[11,17-19]. One case-control study reported a 23.4% emergent colectomy rate in patients with both CDI and IBD (UC) as compared to 13.5% (OR = 2.09, 95%CI: 0.72-6.1; $P = 0.17$) in those with IBD alone^[19]. Another study reported a rate of urgent colectomy as high as 45% in hospitalized patients with IBD colitis and co-existing CDI in 2004, which decreased to 25% in 2005, probably due to changes in the treatment

Table 1 Main short-term outcomes in inflammatory bowel disease patients with *Clostridium difficile* infection as compared to those with inflammatory bowel disease alone or *Clostridium difficile* infection alone

Ref.	Journal and year of publication	Study design and time frame	Outcome
Murthy <i>et al</i> ^[21] Canada	Aliment Pharmacol Ther 2012	In-patients, Ontario, Canada, 2002-2008	Increased LOS (11 d <i>vs</i> 6 d, $P = 0.0001$), similar rate of colectomy (12% <i>vs</i> 9.8%; $P = 0.30$), and higher mortality rate (3.3% <i>vs</i> 0.38%, $P < 0.0001$) as compared with UC patients without CDI
Navaneethan <i>et al</i> ^[24] United States	J Crohns Colitis 2012	Out-/in-patients; 2002-2007	No significant difference in the colectomy risk within 3 months of index admission between UC patients with CDI and those with UC alone
Ananthakrishnan <i>et al</i> ^[28] United States	Aliment Pharmacol Ther 2012	In-patients; 1998-2010	4.4% colectomy and 15.2% mortality rates
Jen <i>et al</i> ^[17] United Kingdom	Aliment Pharmacol Ther 2011	Case-control analysis of United Kingdom Hospital Episodes Statistics, out-/in- patients; 2002-2007	Increased mortality (OR = 6.32), higher risk for surgery (OR = 1.87), and 27.9 d longer LOS than patients with IBD alone
Ananthakrishnan <i>et al</i> ^[18] United States	Inflamm Bowel Dis 2011	Case-control analysis of NIS database, out-/in- patients; 1998, 2004, 2007	Increase in colectomy rate from 1998 (OR = 1.39, 95%CI: 0.81-2.37) to 2007 (OR = 2.51, 95%CI: 1.90-3.34) ($P = 0.03$), and in mortality risk (1998: OR = 2.38, 95%CI: 1.52-3.72) (2007, OR = 3.38, 95%CI: 2.66-4.29) ($P = 0.15$)
Kaneko <i>et al</i> ^[25] Japan	Clin Res Hepatol Gastroenterol 2011	Out-/in-patients; 2006-2009	No association between CDI and colectomy rate in UC patients
Kariv <i>et al</i> ^[20] United States	J Crohns Colitis 2011	Out-/in- patients with UC; 2000-2006	No difference in colectomy rates (48% <i>vs</i> 50.9%, $P = 0.81$) between infected and non-infected UC patients, no mortality in UC patients with or without CDI
Jodorkovsky <i>et al</i> ^[19] United States	Dig Dis Sci 2010	In-patients; 2004/06-2005/06	Similar mean LOS for IBD patients with CDI and those without CDI (11.7 d <i>vs</i> 11.0 d; $P = 0.70$); similar use of cyclosporine therapy (48% <i>vs</i> 47%); higher emergent colectomy rate (23% <i>vs</i> 13.4%, $P = 0.17$)
Bossuyt <i>et al</i> ^[14] Belgium	J Crohns Colitis 2009	In-patients; 2000-2008	LOS shorter as compared to non-IBD patients (15.2 d <i>vs</i> 27.7 d, $P = 0.001$); one patient with UC+ CDI had a semi-urgent colectomy; no mortality in IBD patients, 2 deaths in non-IBD patients
Ricciardi <i>et al</i> ^[15] United States	Dis Colon Rectum 2009	Case-control analysis of NIS database, out-/in- patients; 1993-2003	Increased case fatality in UC+CDI patients but not in those with CD+CDI; operative mortality for UC+CDI patients reached 25.7%
Ben-Horin <i>et al</i> ^[26] Israel and some European countries	Clin Gastroenterol Hepatol 2009	Multi-center, in-patients; 2000-2008	Low colectomy rate (6%) in IBD patients with CDI
Ananthakrishnan <i>et al</i> ^[13] United States	Gut 2008	Case-control analysis of NIS database, out-/in- patients; 2003	Four-fold higher mortality rate (OR = 4.7, 95%CI: 2.9-7.9) compared with IBD alone and twice higher than in those with CDI alone (OR = 2.21, 95%CI: 1.4-3.4); 3-d longer compared with IBD alone; six-fold greater risk of bowel surgery than those with CDI alone (OR = 6.6, 95%CI: 4.7-9.3); 11406 higher hospital adjusted charges
Nguyen <i>et al</i> ^[16] United States	Am J Gastroenterol 2008	Case-control analysis of NIS database, out-/in- patients; 1998-2004	Increased mortality in UC (OR = 3.79, 95%CI: 2.84-5.06) but not in CD patients (OR = 1.66, 95%CI: 0.75-3.66); increased LOS with 65% for CD and 46% for UC, and increased hospital charges compared with non-IBD patients
Rodemann <i>et al</i> ^[12] United States	Clin Gastroenterol Hepatol 2007	In-patients, 1998-2004	LOS similar to non-IBD patients
Issa <i>et al</i> ^[11] United States	Clin Gastroenterol Hepatol 2007	Observational study, out-/ in-patients; 2004-2005	Increased LOS (13 d <i>vs</i> 6 d); the colectomy rate in UC+CDI decreased from 45% in 2004 to 25% in 2005

C. *difficile*: *Clostridium difficile*; CDI: *Clostridium difficile* infection; CD: Crohn's disease; IBD: Inflammatory bowel disease; LOS: Length of hospital stay; NIS: National Inpatient Sample; OR: Odds ratio; UC: Ulcerative colitis.

regimen (use the vancomycin as a primary antibiotic, and a rapid decrease in steroid dosing)^[11]. Using data from NIS, Ananthakrishnan *et al*^[18] reported a significant increase in total colectomy odds from 1998 (OR 1.39, 95%CI: 0.81-2.37) to 2007 (OR = 2.51, 95%CI: 1.90-3.34) ($P = 0.03$) in IBD patients with CDI compared to IBD patients without CDI. Jen *et al*^[17] using HES data for 2002/03 to 2007/08 found that IBD patients with CDI were exposed to a risk of undergoing gastrointestinal surgery or emergency colectomy 1.2 to 3 times higher than those with IBD alone.

In contradiction with previously mentioned studies, others communicated low rates of urgent colectomy in

IBD patients with CDI. Thus, according to two recent studies from Cleveland Clinic's Digestive Disease Institute, United States, CDI in UC patients had no negative impact on colectomy risk within 3 mo of CDI diagnosis. In one study^[20] including 78 patients (39 patients with UC and CDI, 39 with UC alone), 25 underwent colectomy, 12 of whom (48%) were among those with UC and CDI, and 13 (50.9%) with UC alone ($P = 0.81$). Also, in the second study^[24], including 146 patients (45 with UC and CDI, 101 with UC without CDI), within 3 mo of index admission there was no significant difference concerning colectomy risk between UC patients with CDI and those without CDI; however, on long-term follow-up (one

Table 2 Long-term outcomes in inflammatory bowel disease patients with *Clostridium difficile* infection compared to those with inflammatory bowel disease alone

Ref.	Journal and year of publication	Study design and time frame	Outcome
Murthy <i>et al</i> ^[21] Canada	Aliment Pharmacol Ther 2012	In-patients; 2002-2008	UC patients with CDI was associated with increased adjusted 5-yr risk of mortality, but not of colectomy, as compared with UC without CDI
Navaneethan <i>et al</i> ^[24] United States	J Crohns Colitis 2012	Out-/in-patients; 2002-2007	One year following CDI: increased rates of ERV (37.8% <i>vs</i> 4%, <i>P</i> = 0.001) and colectomy (35.6% <i>vs</i> 9.9%, <i>P</i> = 0.001); escalation in medical therapy in 58.8% as compared to the prior year (12.9%) (<i>P</i> = 0.0001)
Jodorkovsky <i>et al</i> ^[19] United States	Dig Dis Sci 2010	In-patients; 2004-2005	One year following CDI: UC patients with CDI had increased rate of ERV (8 <i>vs</i> 1, <i>P</i> = 0.012), higher number of UC-related hospitalizations (58 <i>vs</i> 27, <i>P</i> = 0.001), and two-fold higher rates of colectomy (44.6% <i>vs</i> 25%, <i>P</i> = 0.04) compared to UC alone
Chiplunker <i>et al</i> ^[27] United States	Gastroenterology 2009	Case-control, in-patients; 2005-2006	One year following CDI: over half required an escalation in their IBD medical therapy, 46% had more hospitalisations, colectomy occurred in 10.3% , and no mortality

CDI: *Clostridium difficile* infection; ERV: Emergency room visits; IBD: Inflammatory bowel disease; OR: Odds ratio; UC: Ulcerative colitis.

year), UC patients with CDI showed a significantly higher rate of colectomy than those without CDI. Kaneko *et al*^[25] in a retrospective study from Yokohama City University Medical Center, Japan, reported that CDI did not have an impact on colectomy rate in their hospitalized UC patients with active disease, the difference in colectomy rate between UC+CDI patients (33.6%) and those without CDI (23.1%) being statistically insignificant (OR = 1.03, 95%CI: 0.41-2.63; *P* = 0.94). Two European studies^[14,26] also reported low rates of colectomy (5% and 6%, respectively) in IBD patients co-infected with *C. difficile*. Recently, Murthy *et al*^[21] found a similar rate of colectomy between UC patients with and without CDI (12% *vs* 9.8%, *P* = 0.30).

The different colectomy rates between single-center studies and those using nationwide data could be partially accounted for by differences in healthcare practice and threshold for surgery, response to CDI medical therapy, and data collection methods used^[23].

Mortality rates are higher in IBD patients with CDI than in those without CDI or with CDI alone^[13,16,17,21]. Among IBD patients, mortality is higher in UC than in CD^[16]. All studies analysing nationwide databases reported high rates of mortality in IBD patients with CDI^[13,16,17,21]. Nguyen *et al*^[16] analyzed NIS discharge records from 1998 to 2004 and found that CDI was associated with a nearly four fold increase in mortality among hospitalized patients with UC (OR 3.79, 95% CI: 2.84-5.06) unlike those with CD (OR = 1.66, 95%CI: 0.75-3.66) as compared to non-IBD patients. Similarly, Ananthakrishnan *et al*^[13], using also data from NIS, found that IBD patients with superimposed CDI had a four fold increase in mortality compared to patients hospitalized with IBD alone (OR = 4.7; 95%CI: 2.9-7.9) and twice higher than those with CDI alone (OR = 2.2, 95%CI: 1.4-3.4). In contrast to Nguyen *et al*^[16] study, where mortality was higher only in UC, this study reported an increased mortality rate in both UC and CD patients. Jen *et al*^[17] using HES data, reported that for the studied period (2002-2008), IBD patients with CDI were

approximately six times more likely to die in hospital than those admitted for IBD alone (adjusted OR = 6.32, 95%CI: 5.67-7.04), and suggested that such high mortality rate may be partially due to increased number of all emergency gastrointestinal surgery and colectomy rates during admissions. Murthy *et al*^[21] also reported a higher mortality rate in hospitalized UC patients co-infected with *C. difficile* than in uninfected UC patients (3.3% *vs* 0.38%, *P* = 0.0001).

Nevertheless, other studies showed a mortality rate for IBD patients with CDI similar to or not statistically higher than what was reported for non-infected IBD patients^[14,18]. Thus, Bossuyt *et al*^[14] registered no deaths among their patients with UC and CDI, while Ananthakrishnan *et al*^[18] found a non-significant increase in the relative mortality risk in IBD patients with superimposed CDI from 1998 (OR = 2.38, 95%CI: 1.52-3.72) to 2007 (OR = 3.38, 95%CI: 2.66-4.29; *P* = 0.15).

LONG-TERM OUTCOMES

Few studies reported on long-term outcomes after an initial episode of CDI in IBD patients (Table 2)^[19,21,24,27]. In a retrospective study including 47 patients with UC and CDI and 52 with UC without CDI, Jodorkovsky *et al*^[19] reported that, over the year following the initial infection episode, a significant increase in the number of visits to the emergency room (8 *vs* 1, *P* = 0.012) was registered, as well as a higher number of UC-related hospitalizations (58 *vs* 27, *P* = 0.001) and a two-fold increase in colectomy rate (44% *vs* 25%, OR = 2.38, 95%CI: 1.01-5.6; *P* = 0.04) as compared to UC patients without CDI. Murthy *et al*^[21], in a retrospective cohort study of UC patients with and without CDI, found that CDI was associated with higher adjusted 5-year risk of mortality [adjusted hazard ratio (aHR) = 2.40, 95%CI: 1.37-4.20], but not of colectomy (aHR = 1.18, 95%CI: 0.90-1.54). In another retrospective study^[24], UC patients with CDI had significantly more UC-related emergency room visits (37 *vs* 4, *P* < 0.001) and a higher rate of colectomy (35.6% *vs* 9%, *P* < 0.001).

than those with UC alone in the year following initial infection. In addition, 55.8% of patients with UC and CDI had an escalation in medical therapy in the year after index infection admission as compared to 12.9% in the previous year ($P < 0.0001$). In a multivariate analysis for risk factors of colectomy, severe disease on endoscopy (OR = 16.7, 95%CI: 4.1-67.9; $P < 0.001$) and CDI (OR = 10.0, 95%CI: 2.7-36.3; $P < 0.001$) were found to be independently associated with colectomy within 1 year. Chiplunker *et al*^[27] in a retrospective, case-control study on 81 patients with IBD comparing disease progression 1 year before and 1 year after the initial infection with *C. difficile*, found that 46% of patients had more hospitalizations and over a half of them (53%) required an escalation in medical therapy during the year following CDI. No deaths occurred during the 1 year follow-up.

Healthcare costs are higher in IBD patients with CDI than in those with IBD alone due to longer hospital stay, higher number of IBD-related hospitalizations, increased need for surgery, and hospital care charges^[13,16]. Nguyen *et al*^[16] found a mean cost increased by 46% and 63% for UC and CD patients, respectively, while Ananthakrishnan *et al*^[13] reported higher increased hospital adjusted expenses of US\$ 11406.

DISCUSSION

The majority of published studies (90%) found by searching Medline and other databases aim to assess CDI incidence in IBD patients, while only 10% of them report on outcome following infection. All studies reporting on outcome in IBD patients with concomitant CDI were retrospective, small single-center cohort studies or large nationwide database studies mostly conducted in North America and Europe. Apart from being retrospective, available studies on outcome have several other limitations such as incomplete information on disease severity, absence of reference to *C. difficile* diagnosis, antibiotic and immunomodulatory therapy. It should be underlined that most of analyzed studies relate to hospitalized IBD patients in the early 2000s, when enzyme immunoassay of stool for *C. difficile* toxins A and B has dominated the laboratory diagnosis of CDI, despite its low sensitivity.

Though few in number, studies reporting outcomes associated with CDI in IBD patients most often show that CDI has a negative impact both on short- and long-term IBD outcomes, increasing the need for surgery, morbidity and mortality rates, as well as healthcare costs^[11,13,16-18,21,24,27]. Both major forms of IBD are under increasing risk for CDI, although patients with UC are most susceptible to infection and have more severe outcomes^[15,16,19]. Short-term outcomes, defined as those measured within 30-90 d of index admission, include length of hospital stay, colectomy and mortality rates. Long-term outcomes, measured at least 1 year following index admission, include emergency room visits, UC-related hospitalizations, escalation in medical therapy, colectomy and mortality rates.

Studies report conflicting results on the length of hospital stay in IBD patients with concomitant CDI: some found similar stays^[12,19], others shorter ones^[14], while most of them reported longer hospitalization periods than in IBD patients without CDI or with CDI alone^[11,13,16,17,21]. Similar or shorter hospitalization periods were reported by single-center studies^[12,14,19], while longer stays were found in studies using nationwide databases^[13,16,17,21]. Discrepancies between studies may be explained by disease severity and response to medical therapy in IBD patients included in the analyses.

Contrasting results have also been reported with regard to colectomy rates in IBD patients with CDI^[11,13,14,17-21,24-26]. Thus, some studies analyzing nationwide data (US NIS HCUP, UK HES) reported high colectomy rates^[13,18], while other single-center ones^[20,24,25] found CDI to have no negative impact on colectomy rate in UC patients. There are discrepancies in what concerns colectomy rates even between studies using nationwide data in North America^[13,18] and Europe^[17], probably due to differences in data collection methods and threshold for surgery^[23]. In addition, studies analyzing nationwide data for colectomy also include IBD patients admitted for elective surgery, a fact which can contribute to high colectomy rates^[23]. Lower risks for colectomy, as reported by single-center North-American^[20] and European^[14,26] studies, may be partially explained by CDI prompt response to medical therapy, followed by clinical remission of IBD flare, thus preventing surgery^[20].

Variable results regarding mortality rate in IBD patients with concomitant CDI are also to be noted^[13,14,16-18,21]. High mortality rates reported by some studies using nationwide data^[13,16,17] may be accounting for by to the increased use of colectomy in IBD patients with CDI^[20], and also by the inclusion of all hospitalized patients who presumably have more severe disease than those from cohort studies^[17,23].

Few studies reported long-term outcomes after an initial CDI episode in IBD (UC) patients. Two^[19,24] reported an increased number of visits to the emergency room and in UC-related hospitalizations, and higher colectomy rate than in patients with UC alone in the year following initial infection. UC patients with CDI had an escalation in medical therapy 1 year after index admission as compared to the previous year^[24,27]. Another study including UC hospitalized patients with and without CDI, found that those with associated CDI had a higher adjusted 5-year risk of mortality, but not of colectomy^[21].

We may add that only one study aimed to identify predictive factors for severe outcomes (colectomy, death) associated with CDI in IBD patients^[28]. Ananthakrishnan *et al*^[28] in a retrospective study using multi-institutional electronic medical record database from two large referral hospitals over the period 1998-2010, reported a 4.4% colectomy and 15.2% mortality rates during 180-d follow-up of 294 IBD patients with CDI (mostly with UC), and found that among several demographic variables and laboratory parameters, only serum albumin below 3

g/dL, hemoglobin below 9 g/dL, and serum creatinine above 1.5 mg/dL were independent predictors of severe outcomes^[28].

CDI IN IBD PATIENTS FOLLOWING SURGICAL INTERVENTION

Clostridium difficile enteritis usually occurs in IBD patients who have undergone colonic surgery, mainly proctocolectomy^[29-31]. *C. difficile* enteritis is generally rare, although the number of cases reported in literature has recently increased^[32]. The predisposition of IBD patients with previous colonic surgery to *C. difficile* enteritis may be accounted for by the colonization of the neo-terminal ileum with colonic-type bacterial flora^[33], and phenotypic changes in the epithelium of pelvic ileoanal pouches^[34]. *C. difficile* enteritis diagnosis and treatment are similar to that for colonic CDI. If some studies reported increased mortality among patients with *C. difficile* enteritis^[35], other ones found low or even no mortality^[29].

Clostridium difficile pouchitis has been reported in IBD patients with ileal pouch anal anastomosis (IPAA)^[36-38]. Shen *et al*^[36] found that 18.3% of their 115 patients with IPAA had CDI. Morphologic changes in the pouch epithelium secondary to prolonged exposures to fecal stream may favor CDI^[34]. In addition, frequent antibiotic treatment for acute or chronic pouchitis is another risk factor for CDI in such patients^[36]. Recently, Tyler *et al*^[39] reported that genetic polymorphisms, particularly the NOD2insC risk allele, are associated with increased risk of developing pouch inflammation among patients with UC and IPAA. Treatment with vancomycin, tinidazole, or rifaximin has been used with benefit in many patients^[37,38].

CONCLUSION

IBD patients with CDI are under a higher risk of worse outcomes than those without CDI. Because the available data are often conflicting and obtained from retrospective studies, further prospective multi-center studies are required to evaluate the impact of CDI on IBD outcomes. Until then, to improve patient outcome, clinicians should have a high index of suspicion for CDI in all IBD patients presenting with a disease flare in order to rapidly establish diagnosis and prompt treatment of infection.

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Infectious etiopathogenesis of Crohn's disease

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Abstract

Important advances during the last decade have been made in understanding the complex etiopathogenesis of Crohn's disease (CD). While many gaps in our knowledge still exist, it has been suggested that the etiology of CD is multifactorial including genetic, environmental and infectious factors. The most widely accepted theory states that CD is caused by an aggressive immune response to infectious agents in genetically predisposed individuals. The rise of genome-wide association studies allowed the identification of loci and genetic variants in several components of host innate and adaptive immune responses to microorganisms in the gut, highlighting an implication of intestinal microbiota in CD etiology. Moreover, numerous independent studies reported a dysbiosis, *i.e.*, a modification of intestinal microbiota composition, with an imbalance between the abundance of beneficial and harmful bacteria. Although microorganisms including viruses, yeasts, fungi

and bacteria have been postulated as potential CD pathogens, based on epidemiological, clinicopathological, genetic and experimental evidence, their precise role in this disease is not clearly defined. This review summarizes the current knowledge of the infectious agents associated with an increased risk of developing CD. Therapeutic approaches to modulate the intestinal dysbiosis and to target the putative CD-associated pathogens, as well as their potential mechanisms of action are also discussed.

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Key words: Crohn's disease; Intestinal microbiota; Dysbiosis; Adherent-invasive *Escherichia coli*; Probiotics; Antibiotics; Fecal microbiota transplantation

Core tip: Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract of which the etiopathogenesis is not fully understood. Increasing evidence has shown that the etiology of CD is multifactorial involving genetic, environmental and infectious factors. A dysbiosis with an increase in the abundance of putative pathogenic bacteria and a decrease in that of potentially beneficial bacteria has been observed in CD patients, revealing the involvement of intestinal microbiota in such disease. This review aims to summarize the current knowledge of the infectious etiology of CD and to discuss therapeutic approaches to modulate intestinal dysbiosis and to target CD-associated pathogens.

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INTRODUCTION

The etiopathogenesis of Crohn's disease (CD), a type of inflammatory bowel diseases (IBD), is complex and con-

sists, according to clinical and epidemiological studies, of three interacting elements: environmental factors, genetic susceptibility and infectious agents. While many gaps in our knowledge still exist, the most widely accepted theory holds that the disorder is caused by an aggressive immune response to microorganisms of the intestinal microbiota in genetically predisposed individuals.

The early identification of nucleotide-binding oligomerization domain-containing protein 2 (NOD2), an intracellular sensor of pathogen/microbe-associated molecular patterns, as a susceptibility gene for CD^[1,2] has highlighted the role of innate immunity in the disease. This has been substantiated by genome-wide association studies (GWAS) with the identification of genetic association between CD susceptibility and variants in genes involved in autophagy, ATG16L1 (autophagy-related 16-like 1) and IRGM (Immune-Related GTPase M)^[3-6]. To date, 70 independent loci or genetic variants linked to various components of innate and adaptive immunity have been identified by GWAS as CD susceptibility factors^[4-12]. Arguments in favor of the involvement of environmental factors in CD etiology, which are based on the observation of the irregular distribution of CD cases worldwide, have been raised. Since the appearance of IBD in the middle of the 20th century, the CD incidence and prevalence have shown a continually growing profile in industrialized countries or “Western” countries, such as North Europe and North America, suggesting an involvement of lifestyle^[13]. Another epidemiological study also showed that “Western” diet, rich in fat and sugar and poor in fibers, is associated with an increased risk of developing CD^[14]. This is recently reported that active smoking is associated with an increased risk of developing CD and that smoking cessation leads to a reduced progression of the disease comparatively to patients who still smoke^[15]. Other environmental factors, such as antibiotic use, social status, microbial exposure early in life and during life have been also associated with CD^[16]. Whether these factors, along with genetic susceptibility, lead directly to CD, or whether they allow the conditions needed for infectious agents to thrive, is not clear.

Numerous epidemiological studies, clinicopathological data, genetic and experimental evidence increasingly support an implication of microorganisms in CD pathogenesis. Three non-mutually exclusive theories are currently explored to explain the infectious etiology of CD: (1) a “dysbiosis”, *i.e.* a modification of intestinal microbiota composition with an imbalance between beneficial and harmful bacteria; (2) an excessive bacterial translocation caused by a disrupted intestinal barrier function and defective immune responses; and (3) persistence of a pathogen. This review summarizes our current knowledge of various organisms that have been postulated as infectious agents in CD, and discusses how this may be relevant to the pathogenesis of CD and the new therapeutic approaches.

INTESTINAL MICROBIOTA AND CD

Human intestinal microbiota

The human gastrointestinal (GI) tract contains 10¹⁴ microorganisms of more than 500-1000 different species, forming intestinal microbiota^[17]. The density of intestinal microbiota varies along the GI tract, going from 10² colony forming units (CFU) per gram in stomach to 10¹² CFU per gram in colon^[18].

The intestinal microbial composition can vary greatly between individuals, and an epidemiological study comparing the fecal microbiota between African and European children showed that its composition is determined in part by hygiene, geography and diet^[19]. Higher similarity in fecal bacterial species was reported within twins than in genetically unrelated couples sharing environment and dietary habits^[20]. The gut microbiota composition of siblings also showed increased similarity compared to that of spouses, who were living in the same environment and had similar eating habits^[21].

Given the complexity of the human intestinal microbiota, the characterization of its composition using conventional culture methods and morphological and biochemical-based traditional techniques is limited. Development of new biomolecular techniques, using high-throughput sequencing, allows circumventing these difficulties. Two approaches are currently available. The first is based on sequencing of the 16S ribosomal RNA coding gene (16S rDNA), which is conserved between all phylogenetic bacterial groups^[22]. The second one, namely the metagenomic approach, is based on a complete sequencing of bacterial genome. The evolution of high-throughput technologies with next-generation sequencing allows producing thousands or millions of sequences at once, reducing drastically the costs and facilitating access to full metagenomic sequencing. Dominant bacterial populations in the human intestinal microbiota (> 90%) belong to two phyla: the Firmicutes and the Bacteroidetes; the remainders belong to rarer phyla such as Proteobacteria (containing genera such as *Escherichia* and *Helicobacter*) and Actinobacteria as well as viruses, protists, and fungi^[23-26]. Interestingly, mucosa-associated microbiota is different from the fecal microbiota^[27]. The composition of the fecal microbiota may temporally vary following exposure to different types of foods, medications, or physical environments, and also from changes in transit time, as microbial composition in the lumen varies from caecum to rectum^[24].

Intestinal dysbiosis and CD

An imbalance of the intestinal microbiota, *i.e.* a modification of its composition, with decreased complexity of commensal bacterial profiles and higher numbers of mucosa-associated bacteria, has been reported in CD patients.

Using a 16S rDNA-based profiling technique, Ott

and colleagues showed that the diversity of mucosa-associated microbiota in specimens from patients with active CD undergoing surgery was markedly reduced compared with mucosal specimens from control individuals without inflammation^[28]. Metagenomic studies have shown a decrease in the abundance of several species of the Firmicutes and the Bacteroidetes phyla in CD patients compared with control subjects^[29-32]. The decrease in the abundance of Bacteroidetes could contribute to inflammation since some bacteria belonging to this phyla such as *Bacteroides fragilis* have been shown to exhibit protective effects in a mouse model of colitis induced by *Helicobacter hepaticus*, a murine commensal bacterium with pathogenic properties^[33]. Among Firmicutes, a decrease of the amount of *Faecalibacterium prausnitzii* (*F. prausnitzii*) has been observed in CD patients compared with control subjects^[34]. In mouse models of intestinal inflammation, administration of *F. prausnitzii* resulted in anti-inflammatory effects^[34]. Therefore, the decreased abundance of *F. prausnitzii* could contribute to intestinal inflammation in CD. It has been consistently reported that CD patients have relatively increased amount of Enterobacteriaceae, particularly *Escherichia coli* (*E. coli*) species, compared with control subjects, with a more pronounced difference was observed for mucosa-associated microbiota than fecal samples^[35-43]. An increase in the abundance of some mucolytic bacteria, such as *Ruminococcus gnavus* and *Ruminococcus torques*, in CD patients was also observed^[44].

ROLE OF BACTERIA IN THE PATHOGENESIS OF CD

The intestinal mucosal surface is in a continuous contact with the intestinal microbiota. Given the enormous numbers of enteric bacteria and the persistent threat of opportunistic invasion, it is crucial that the host maintains homeostasis at the luminal surface of the intestinal-microbial interface. This is mediated by a perfect integrity of the intestinal barrier and a functional immunotolerance to the intestinal microbiota and luminal antigens.

Excessive bacterial translocation caused by intestinal epithelial barrier dysfunction

The intestinal barrier allows the absorption of water, ions and nutrients without leaving the microorganisms to penetrate across the mucosal surface. The first line of defence between the intestinal lumen and inner milieu, the physical barrier, is made up of a layer of columnar epithelial cells. More than 80% of these cells are enterocytes, and the rest are enteroendocrine, goblet, and Paneth cells^[45]. Epithelial cells are connected *via* the intercellular junctional complexes including tight junctions, adherent junctions, desmosomes and gap junctions^[46].

Many studies have shown an increased intestinal permeability in CD patients during active phases and a decreased permeability in remission phases^[47-51]. Electron microscopy analyses of biopsies from CD patients in active phases revealed a reduced number of tight junctions

compared with control subjects^[52]. A deregulation of tight junction proteins has been reported in CD patients, with an up-regulation of claudin-2 and a down-regulation of claudin-5 and 8^[52]. The alteration of intestinal permeability observed during active phases of CD could explain the chronic inflammation, given the probably resulting transit of bacteria and other luminal antigens through the mucosa, which are able to activate the sub-mucosal innate immune system.

The intestinal epithelial surface is covered by a mucus layer that prevents the contact between the epithelial layer and microorganisms and the diffusion of unwanted substances, as well as protects the physical barrier from sheer stress. The main component of the mucus layer is mucins secreted by goblet cells, which are heavily glycosylated proteins^[53]. The outer loose mucus layer contains a limited number of intestinal microbes; whereas the inner adherent mucus layer contains very few microbes, forming a protected zone adjacent to the epithelial surface^[54]. It is likely that the antimicrobial proteins, which are secreted by epithelial cells and are retained in the mucus layer, contribute to the maintenance of low bacterial numbers in the inner mucus layer^[55]. These “bodyguards” are members of several distinct protein families such as defensins, cathelicidins, and C-type lectins, and they promote bacterial killing by targeting the integrity of bacterial cell walls^[56]. Mice lacking the mucin MUC2 are unable to maintain this relative “bacteria-free” zone and suffer from intestinal inflammation^[54]. It has been shown that mucin gene expression, mucus composition and secretion are altered by intestinal microbiota and host-derived inflammatory mediators^[53].

Dysfunction of immunotolerance and innate immune response to bacteria

Maintenance of immunotolerance and innate immune responses, which allows the control of inflammatory responses in intestinal epithelium, is mediated by several mechanisms: (1) secretion of IgA; (2) bacterial clearance *via* the production of antimicrobial peptides; or (3) a functional autophagic process. Changes in these processes have been observed in CD, which could contribute to abnormal immune responses.

Defective secretory IgA production in CD: The IgA immunoglobulins are secreted by B lymphocytes localized in the intestinal lamina propria^[57]. The secretory IgA is transcytosed across the epithelium and retained in the mucus layer, where it acts to entrap the luminal antigens and bacteria. Bacteria present in the lumen or penetrating the intestinal epithelium are detected by dendritic cells that will alert B cells in the Peyer's patches, which will, in turn, produce IgA specific for intestinal bacteria^[57]. Mice that lack activation-induced cytidine deaminase (AID), which results in defective IgA production in the intestine, exhibit an expansion of mucosa-associated bacteria such as segmented filamentous bacteria (SFB)^[58]. This suggests that secreted IgA also regulates the composition and

density of bacterial communities^[58]. In IBD patients, a serologic shift from an IgA-dominant to an IgG-dominant response in the intestine, which may act as another local defense line, has been reported^[59]. IgG is likely to have an inflammatory effect because in response to flagellin, a common bacterial antigen, the neonatal receptor for IgG FcRn, expressed in hematopoietic cells, promotes inflammation in the presence of anti-flagellin IgG in mice^[60].

Defective bacterial killing through secretion of anti-microbial peptides: The intestinal epithelia secrete anti-microbial molecules whose function is to kill commensal or pathogenic bacteria. Among these molecules are peptides named defensins. Most defensins function by binding to the microbial cell membrane, and, once embedded, forming pore-like membrane defects that allow efflux of essential ions and nutrients^[61,62]. Two classes of defensins have been described in human, α and β -defensins. The α -defensin peptides are mainly secreted by Paneth cells and neutrophils, while β -defensins are more generally secreted by epithelial cells^[61]. The biosynthesis of defensins is triggered by the activation of receptors involved in recognition of extracellular and intracellular bacterial components like Toll-like receptors (TLR) and NOD receptors, respectively, leading to a rapid killing of bacteria in contact with the intestinal epithelium^[63]. Changes in intestinal microbiota were observed in mice that express the human α -defensin 5 and also in mice that do not produce functional α -defensins^[64], suggesting that defensins also regulate the composition and density of bacterial communities. A decrease in α -defensin expression in Paneth cells has been reported in patients with ileal CD, particularly those carrying mutations in *NOD2* gene^[65], indicating the link between infectious etiology and host genetic susceptibility. Reduced expression of β -defensins has been observed in patients with colonic CD^[65]. Other antimicrobial proteins including lysozyme and RegIII γ are secreted by Paneth cells upon exposure to bacteria or bacterial antigens^[66], thereby contributing to host defense against mucosal penetration of both symbiotic and pathogenic bacteria. Mice with a genetic ablation of Paneth cells exhibit increased translocation of bacteria into the host tissues, indicating that Paneth cells contribute to maintaining luminal compartmentalization of intestinal bacteria^[67]. The abnormal synthesis of antimicrobial proteins in CD patients could result in increased intestinal barrier permeability to bacteria that could consequently lead to chronic inflammation.

Defective bacterial clearance by autophagy: Autophagy is a homeostatic process that involves degradation of dysfunctional cellular components through the lysosomal machinery. The newly discovered specialized role of autophagy expands autophagic functions as an immune defense mechanism against intracellular pathogens (also referred to as xenophagy)^[67,68]. GWAS have revealed CD-associated risk variants in several autophagy genes, such as *ATG16L1*, *IRGM*, *ULK1* (Unc-51 like

autophagy activating kinase 1), *PTPN2* (protein tyrosine phosphatase nonreceptor type 2) and *LRRK2* (leucine-rich repeat kinase 2)^[68]. This raised autophagy as one of the most attractive molecular pathways in the field of CD. Further efforts have been made to investigate a functional implication of autophagy in CD pathogenesis^[68,69]. A link between autophagy and the innate immune receptor NOD2 has been established, the latter recruits and interacts with ATG16L1 at site of bacterial entry in the plasma membrane^[70-72]. These studies have also shown that in epithelial cells, macrophages and dendritic cells, one of the *ATG16L1* or *NOD2* risk variants could result in impaired intracellular pathogenic bacterial clearance owing to a defect in xenophagy response. CD patients homozygous for the *ATG16L1* risk allele exhibited structural aberrances in Paneth cells similar to those observed in mice with hypomorphic ATG16L1 expression, *i.e.* decreased granule number and lack of lysosomes in the ileal mucus layer^[73]. This indicates that defects in intestinal barrier function in CD could involve dysfunction of Paneth cells related to *ATG16L1* mutation. Interestingly, the CD-associated c.313C>T polymorphism located within the *IRGM* mRNA region results in loss of binding of microRNA-196^[74]. This consequently leads to aberrance of regulation of *IRGM* expression by microRNA-196 and defects in autophagy-mediated control of intracellular replication of the CD-associated adherent-invasive *E. coli*^[74]. Together, these studies suggest that a defect in the autophagy machinery in CD patients could lead to an uncontrolled bacterial proliferation inside host cells and consequently cause chronic inflammation.

INFECTIOUS AGENTS AND CD

Numerous epidemiological, clinicopathological, genetic and experimental evidence has suggested an intervention of infectious agents in CD etiology. Firstly, the preferential location of the lesions in CD are situated in the terminal ileum and colon^[75], where the largest population of bacteria is found^[18]. Secondly, the use of antibiotics in CD treatment has been proven to be sometimes effective^[76]. Thirdly, higher numbers of mucosa-associated and internalized bacteria in biopsies from CD patients compared to control subjects was also reported^[57]. These observations, together with the identification of CD-associated polymorphisms in genes encoding innate immune receptors involved in the recognition of bacterial components or proteins participating in the clearance of pathogenic bacteria by autophagy highly support the hypothesis of an involvement of infectious agents in CD etiology. Those who have been suspected to modify the risk of developing CD include viruses, eukaryotes and bacteria.

Implication of virus in CD

Investigations of viral agents in CD patients have been accomplished with the use of PCR and RT-PCR, and allowed to identify the Epstein Barr virus (EBV) in

15% of patients^[77]. No enterovirus has been detected in the gut of CD patients^[77]. Interestingly, Cadwell *et al*^[78] showed that the abnormalities of Paneth cells in hypomorphic ATG16L1tm mice are dependent on a contact with a particular murine norovirus strain CR6, since mice raised in a germ-free condition or mice infected with a non-persistent norovirus strain exhibited normal Paneth cell morphology. In humans, several clinical studies have shown that norovirus infection can aggravate IBD symptoms^[79,80]. Although there is no direct evidence showing that viral infection could be a causative factor of CD, the study by Cadwell *et al*^[78] suggests that the combination of host genetic susceptibility and the presence of viral factors could lead to CD occurrence.

Bacteriophages are other viral agents that have been suspected to play a role in CD pathogenesis. Indeed, it has been shown that bacteriophages may result in dysbiosis by triggering a destabilization of microbial communities^[81]. A study analyzing the bacteriophage population in CD patients reported that each patient is colonized by one dominant phage family^[82]. In addition, the amount of bacteriophages is significantly increased in CD patients compared with control subjects, and is decreased in ulcerated areas compared with non-ulcerated areas^[82].

Implication of yeast in CD: *Candida albicans*

In 2006, the presence of anti-*Saccharomyces cerevisiae* antibodies (ASCA), involved in the recognition of a mannose residue on the surface of the non-pathogenic yeast *Saccharomyces cerevisiae*^[83], was shown in the serum of 39%-70% of CD patients *vs* 0%-5% of control subjects^[84]. A study proposed that the fungal pathogen *Candida albicans* could act as an intestinal pathogen by triggering the production of ASCA, given that it expresses the ASCA epitope on many surface molecules^[85]. The presence of ASCA in CD patients could reflect a decrease of immunotolerance towards specific antigens of this endogenous yeast. It has been observed that CD patients and their unaffected relatives display a greater colonization of the gastrointestinal tract by *Candida albicans* with respective values of 44 and 38% than the general population with 22%^[86]. In addition, *Candida albicans* colonizes and aggravates gut inflammation in mice^[87]. Although its role in CD etiopathogenesis has not yet been elucidated, the hypothesis of an involvement of *Candida albicans* needs to be taken into consideration.

Implication of pathogenic bacteria in CD

***Mycobacterium avium* subspecies *paratuberculosis*:** *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the causative agent of the Johne's disease, a chronic granulomatous ileitis most common in ruminants, but can also affect many other species including primates. Given that this pathology shares some facets with CD, MAP could be an agent implicated in the complex etiology of CD^[88,89]. Research groups aiming to identify MAP in CD patients by isolation methods or by amplification of specific DNA sequences have reported contradictory re-

sults; while some show the presence of this bacterium in the blood and intestinal biopsies from CD patients^[90-94], some do not^[95-98]. Furthermore, serologic analyses have highlighted the presence of antibodies against MAP in 90% of CD patients^[99]. Administration of antibiotics with strong activity against mycobacteria has resulted in remission in approximately 66%-75% of patients with active CD as reported by three independent studies^[100-102]. Although these antibiotics are also active against other bacterial groups and their effect needs to be confirmed, these studies highlight the potential role of MAP in CD etiology.

***Yersinia*:** Yersiniosis, an infectious disease caused by the psychrotrophic bacterium *Yersinia*, displays the common facets of CD, including the presence of granulomas and ulcerations along the epithelium^[103]. Another study has shown the penetration of *Yersinia enterocolitica* across the epithelium *via* Peyer's patches^[104]. A *Yersinia enterocolitica* oral infection induces the secretion of pro-inflammatory cytokines in mice^[105]. The presence of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* strains in the gut of CD patients has been shown^[106,107]. It was also reported that two cases of patients displaying terminal ileitis involving *Yersinia paratuberculosis* were diagnosed with CD thereafter^[108,109]. These observations support the hypothesis of the involvement of *Yersinia* in CD pathogenesis, but further studies are required to determine their precise role.

***Listeria*:** Numerous studies have been conducted to investigate the role of *Listeria* in CD etiology^[110,111]. Immunohistochemical^[112] and molecular^[111,113] analyses have shown the presence of *Listeria monocytogenes* in CD lesions. *Listeria monocytogenes* has been shown to disrupt and cross the intestinal barrier by entering nonphagocytic cells, escaping from the internalization vacuole, allowing bacteria to move in the cell and to spread from cell to cell^[114]. A study reported that NOD2-deficient mice display an increased susceptibility to oral infection by *Listeria monocytogenes*, with a down-regulation of genes coding cryptids, the murine homologs to human α -defensins, in Paneth cells^[115]. These elements are in favor of the hypothesis that *Listeria* is involved in CD etiology, but additional studies are required to ascertain its causative role.

***Helicobacter*:** Bacteria belonging to the *Helicobacter* family have been suspected to play a role in CD pathogenesis. An association between the *Helicobacter pylori* strain and the human gastric mucosal system was highlighted since *Helicobacter pylori* provokes mucosal ulcerations^[116]. Numerous species of *Helicobacter* have been identified in the human gut^[117,118], suggesting that they can cause pathology by colonizing the intestinal mucosa. *In vivo* studies have shown that *Helicobacter hepaticus*, a benign murine commensal bacterium closed to the human *Helicobacter pylori* strain, was able to induce considerable intestinal inflammation in immunocompromised mouse models [mice deficient in T-cell receptor alpha, T-cell receptor

beta or interleukine (IL)-10] by triggering similar immune responses to those observed in CD^[119,120]. These experimental data suggest that *Helicobacter* could initiate disease in individuals being genetically susceptible to CD.

E. COLI AND CD

The involvement of *E. coli* in CD etiopathogenesis has been argued for long time. According to serologic studies, the antibodies raised against the outer membrane porin C of *E. coli* (anti-OmpC) have been found in 37%-55% of CD patients^[121,122]. Numerous studies have shown the presence of *E. coli*-specific antigens in biopsies from CD patients, particularly in the ulcer areas, along the fissures and within the granulomas and lamina propria^[37,112,123,124]. These reports are in accordance with numerous independent studies showing increased abundance of *E. coli* in the mucosa-associated microbiota of CD patients with dysbiosis compared with control subjects^[37-43]. Specifically, we have shown that *E. coli* abnormally colonize acute and chronic ileal lesions of CD patients comparatively to control subjects^[35,36].

Pathogenic traits of CD-associated *E. coli*

Adhesion and invasion of epithelial cells: Phenotypic characterization of the *E. coli* strains isolated from CD patients has evidenced their capacity to adhere to eukaryotic cells *in vitro*. It has been shown that 53%-62% of CD patients carry *E. coli* strains that display adhesion properties to buccal cells *vs* only 5%-6% of control subjects^[125,126]. Another study reported that 84.6% of CD patients and 78.9% of patients with disease recurrence carry *E. coli* strains capable of adhering to human intestinal epithelial Caco-2 cells, *vs* only 33.3% of control individuals^[35]. Finally, several independent studies have shown the presence of *E. coli* strains internalized in the intestinal mucosa of CD patients and their capacity to invade intestinal epithelial cells (IECs)^[36,39,41,42,127]. Our group has more particularly studied the *E. coli* reference strain LF82, isolated from a chronic ileal lesion of a CD patient^[35,36], and shown that LF82 is able to adhere to and to invade IECs^[128].

Survival and proliferation in host cells: Increasing evidence has shown the capability of the CD-associated *E. coli* strains to invade, survive and replicate in IECs and macrophages. The first study showed by electron microscopy that the *E. coli* strain LF82 can trigger, in the same way as other enteropathogens such as *Shigella*, the lysis of endocytic vacuoles to be released in the cytoplasm, where the environment is more favorable for bacterial replication^[129]. It has been later reported that CD-associated *E. coli* are able to survive and replicate in macrophages without inducing cell death^[130,131]. The mechanism underlying these pathogenic properties of CD-associated *E. coli* has been then investigated. Given the association of polymorphisms in autophagy genes *ATG16L1* and *IRGM* with an increased risk of developing CD, it has been proposed that defects in autophagic process could allow

the CD-associated *E. coli* to survive and replicate within host cells. Our group has shown that the *E. coli* strain LF82 replicates more importantly in autophagy-deficient murine fibroblasts than in wild-type fibroblasts, and in human epithelial cells and macrophages with siRNA-mediated *ATG16L1* expression silencing^[132]. Increased intracellular replication of the LF82 strain was also observed in human cells expressing the *ATG16L1* risk variant^[132]. As discussed earlier, the CD-associated C313T mutation in *IRGM* gene results in loss of tight regulation of *IRGM* protein and therefore autophagy, leading to an increased persistence of the LF82 bacteria in host cells^[174]. These studies suggest that impaired capacity of autophagy to handle and clear bacteria could be a mechanism underlying the increased risk of CD patients *via* increased numbers of pro-inflammatory bacteria.

Disruption of the intestinal barrier function: Several pathogenic bacteria are capable of disrupting the intestinal barrier to cross the mucosal surface by modulating expression and/or organization of proteins involved in establishment and maintenance of epithelial cell junctions. It has been shown that the CD-associated *E. coli* strains induce disorganization of F-actin and displacement of ZO-1 and E-cadherin from the apical junctional complex in human intestinal Caco-2 cell monolayer, leading to a drop in the trans-epithelial resistance and consequently increased epithelial permeability^[127]. Likewise, the LF82 strain induces a redistribution of ZO-1 in Madin-Darby canine kidney-1 cell monolayer, causing a severe disruption of the epithelial barrier^[133]. These data suggest that the CD-associated *E. coli* could play a causative role in CD etiopathogenesis by inducing disruption of intestinal barrier function.

Inducing pro-inflammatory cytokine/chemokine production:

Several *in vitro* and *in vivo* studies have reported that the CD-associated *E. coli* can induce pro-inflammatory responses in host cells. Infection of macrophages with the LF82 strain induces the secretion of high level of TNF- α ^[127,130], and this is essential for the intramacrophagic replication of the bacteria^[130]. This indicates that the CD-associated *E. coli* could induce production of TNF- α to create an amplification loop of replication and of inflammation. Increased production of IL-8 in LF82-infected IECs has been also reported^[127,134,135]. In a transgenic CEABAC10 mouse model expressing human Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), the CD-associated *E. coli* strain LF82 can induce a severe colitis accompanied with an increase in production of the pro-inflammatory cytokines IL-1 β , IL-6, and IL-17 and a decrease in that of the anti-inflammatory cytokine IL-10^[136]. These *in vitro* and *in vivo* data support the hypothesis of the involvement of CD-associated *E. coli* in the etiopathogenesis of this chronic inflammatory disease.

Adherent-invasive *E. coli*: A new pathovar

Pathovar definition: Analysis of virulence factors and

clinical manifestations engendered by different *E. coli* strains has allowed distinguishing six pathovars: enterotoxigenic *E. coli*, enterohemorrhagic *E. coli*, enteroaggregative *E. coli*, diffusely adherent *E. coli*, enteropathogenic *E. coli* and enteroinvasive *E. coli* (EIEC)^[137]. CD-associated *E. coli* strains share some virulence features with already established *E. coli* pathovars such as the ability to induce macrophage cell death, but the factors involved in the adhesion and invasion properties of the known pathovars are not present in the CD-associated *E. coli* strains^[35,129]. Thus, a new pathovar was defined to classify these strains, and called adherent-invasive *E. coli* (AIEC)^[129]. The criteria of this pathovar group include abilities to adhere to and to invade IECs, to survive and replicate in large vacuoles within macrophages without inducing cell death, and to induce secretion of high levels of the pro-inflammatory cytokine TNF- α by infected macrophages. The ability to trigger increased intestinal permeability also constitutes one of the pathogenic characteristics of AIEC^[127]. Finally, AIEC have been shown to form biofilm and to induce granulomas formation *in vitro*^[138-140]. The *E. coli* LF82 strain displays all of these characteristics, and is therefore considered as the AIEC reference strain.

AIEC prevalence in CD patients: Evidence has shown a high prevalence of ileal mucosa-associated AIEC in CD, since AIEC have been identified in the neoterminal ileum of 36.4%-51.9% of CD patients *vs* only 6.2%-16.7% of controls^[36,141]. Comparative genomic analyses of AIEC strains isolated from different patients have shown that only one specific strain was not found in all of the patients, nevertheless, some genotypes of particular strains seem to be more frequently associated with ileal lesions of CD^[40,142].

Virulence factors of AIEC: Genetic determinants of virulence of the AIEC reference strain LF82 are not known and are not similar to those of other invasive *E. coli* strains. Thus, they have been searched by random mutagenesis (insertion of the transposon *Tn5phoA*) and by comparison of the genome of LF82 with that of other pathogens^[143,144]. These studies have permitted the identification of the lipoprotein NlpI which appears to be involved in adhesion and invasion capacities of LF82, since the insertion of the *Tn5phoA* transposon in the NlpI-encoding gene leads to a loss of invasion capacity of LF82 and the LF82- Δ *nlpI* isogenic mutant showed a decreased adhesion and invasion capacity in Intestine-407 epithelial cells^[145]. Likewise, the analysis of the *Tn5phoA* insertion mutant library and the construction of isogenic mutants led to the identification of flagella and the membrane proteins YfgL, OmpC and OmpA as factors involved in adhesion and invasion properties of the reference strain LF82^[146-149]. Another study showed that type 1 pili are a crucial virulence factor that allows AIEC to adhere to IECs *via* the receptor CEACAM6^[150]. They are composed of a major subunit with repetition

of FimA protein, minor subunits FimG and FimF, and one adhesin called FimH present at the end of the pilus^[151]. Our group recently showed that point mutations in FimH confer AIEC bacteria a higher ability to adhere to CEACAM6-expressing human IECs^[152]. The replacement of FimH-coding gene having an AIEC-associated mutation in the LF82 strain by a gene coding FimH of the commensal non-pathogenic *E. coli* K12 MG1655 strain decreased the ability of the bacteria to colonize the gut and to induce intestinal inflammation in CEABAC10 transgenic mice^[152]. This suggests that selection of amino acid mutations in FimH is a mechanism of AIEC virulence evolution, which could increase the risk of CD development in a genetically susceptible host. Recently, we have identified long polar fimbriae (LPF) as a key factor for AIEC to target microfold cells (or M cells) on the surface of Peyer's patches, and that the prevalence of the AIEC strains harboring the *lpf* operon was markedly higher in CD patients compared with controls^[153]. This operon has also been identified in other enteropathogenic bacteria such as *Salmonella* Typhimurium and been shown to be involved in specific adherence of the bacteria to M cells on Peyer's patches^[154]. Interestingly, bile salts, of which the composition has been reported to be modified in CD patients^[155], induce LPF expression favoring the colonization of the epithelium by AIEC^[156]. These data could explain the presence of early lesions in the Peyer's patches of CD patients.

THERAPEUTIC APPROACHES

TARGETING INFECTIOUS AGENTS TO TREAT CD

Current CD treatment strategies aim to control inflammation, relieve symptoms and correct nutritional deficiencies. The treatment depends on the location and severity of disease, complications and response to previous treatment. At this time, treatment can help control the disease, but there is no cure. Established therapies for CD include anti-inflammatory agents [*e.g.*, aminosalicylates (5-ASA), omega 3 fatty acids], immunosuppressive drugs (*e.g.*, corticosteroids, azathioprine and 6-mercaptopurine) and antibiotics. An increasing number of novel and alternative therapeutic approaches are in progress^[157]. New biologic therapies include the targeting of pro-inflammatory cytokines, enhancement or infusion of anti-inflammatory cytokines, blocking intravascular adhesion molecules, and modifying T-cell functions^[157]. Given the increasing evidence supporting the infectious etiology of CD, therapeutic approaches to manipulate gut microbiota have been attempted by using antibiotics, probiotics, prebiotics and possibly defensins. Although these approaches are widely used, their benefits are variable and certainly not permanent. One important reason for this is the fact that the etiology of CD is complex and multifactorial, and does not include only infectious factors. Therefore, manipulation of the gut microbiota is beneficial, but, on its

own, is insufficient to cure the disease.

Antibiotics

The beneficial effect of broad-spectrum antibiotics in the treatment of a moderate form of CD has been reported, although it lacked a large-scale clinical trial^[158]. A controlled clinical trial conducted in American and Canadian centers reported that metronidazole, an antibiotic active against strictly anaerobic bacteria, is more effective in CD patients than a placebo at both a low dose (10 mg/kg per day) and a high dose (20 mg/kg per day)^[159]. A therapy based on ciprofloxacin has been shown to be effective in CD treatment and is also effective in combination with conventional treatments in patients with resistant CD^[160,161]. Combination of ciprofloxacin and metronidazole has been tested in treatment of acute phase of the disease and appeared to be effective^[162]. Numerous clinical trials have been performed to test the potential benefit of antibiotics during different clinical manifestations of CD. Papi and colleagues showed that administration of antibiotics (metronidazole and ornidazole) is effective in preventing post-operative recurrence of CD, which is inevitable since the surgery is not curative^[163]. The efficiency of antibiotics in the treatment of perianal fistulas, a complication of CD, was tested, but did not allow obtaining extended closure of fistulas^[164]. The authors of this study suggest the use of antibiotics as a second-line therapy for fistula healing following the use of anti-TNF- α antibodies, which are known to be effective. Pre-operative administration of antibiotics seems to reduce the risk of surgery^[165]. Although antibiotic treatment is effective in some cases, it has some side effects including non-specific effects against microbiota, the possibility of inducing an antibiotic resistance and the risk of *Clostridium difficile* superinfection. Those antibiotics have been therefore recommended as a second-line treatment for CD.

Probiotics

Given that intestinal dysbiosis has been postulated to cause CD in genetically predisposed individuals, therapeutic strategies based on the use of probiotics have been developed to modulate the imbalance of intestinal microbiota observed in CD patients.

Potential action mechanisms of probiotics include competitive interactions with enteropathogens, production of antimicrobial metabolites, influences on the epithelium, and immune modulation^[166]. The use of the probiotic yeast strain *Saccharomyces boulardii* has been shown to be effective in prevention and treatment of antibiotic-associated and *Clostridium difficile* infection-associated diarrhea, as well as traveler's diarrhea^[167]. Several probiotic strains have been tested in CD treatment. Treatment of CD patients with the probiotic *E. coli* strain Nissle 1917 leads to a remission more rapidly than untreated patients, without affecting the number of patients entering remission^[168]. One study, although involving only a few subjects, 32 patients, reported the maintenance of remission

in CD patients treated with the probiotic strain *Saccharomyces boulardii* comparatively to patients treated with mesalamine^[169], of which the effect in maintaining remission has been raised^[170,171]. However, a recent randomized, placebo-controlled trial reported no significant effect of the yeast *Saccharomyces boulardii* in preventing relapse following a medically-induced remission^[172]. Clinical trials have been carried out to evaluate the potential efficacy of the probiotic strain *Lactobacillus GG* in the prevention of post-operative recurrence in CD patients^[173] and on the average time of relapse after a medically induced remission period^[174]. The first reported contradictory effects with rates of clinical and endoscopic recurrence of 16.6% and 60%, respectively, in the *Lactobacillus*-treated group *vs* 10.5% and 35.3% in the placebo group. The second showed a shorter average time of relapse of 9.8 mo in patients treated with the probiotic *vs* 11 mo in the placebo group. Another strain of *Lactobacillus*, *Lactobacillus johnsonii*, was tested in CD patients during two double-blind trials, and both reported no significant effect of this strain in preventing clinical recurrence of the disease following a surgically-induced remission in probiotic-treated patients comparatively to the placebo group^[175,176]. Although probiotics may be the most physiologic and non-toxic way to prevent and treat CD, it may be transient and has a limited and debatable usefulness at present.

Fecal microbiota transplantation

Given the potential role played by intestinal microbiota in CD pathogenesis, another therapeutic approach has been considered for CD treatment: fecal microbiota transplantation. The transfer of fecal microbiota from a healthy individual to the gut of a patient, enabling the re-establishment of a normal microbial community, has been shown to be effective in the treatment of ulcerative colitis^[177], another form of IBD, or infection with *Clostridium difficile*^[178]. *Clostridium difficile* infection has become a major public health problem, occurring after antibiotic treatment or ingestion of spores in the environment. In patients with a recalcitrant infection, fecal microbiota transplantation has been shown to be effective, with an efficiency rate of 90%^[179,180]. Only few case reports and case series of fecal microbiota transplantation for the management of CD have been published. The first case was a 31-year-old man diagnosed with terminal ileal CD who remained symptom-free for 4 mo after the transplantation^[181]. Among the other cases reported, the use of fecal microbiota transplants leads to CD resolution, *i.e.* to a complete cessation of symptoms or to the absence of active disease confirmed by endoscopic and histologic analyses, but in most cases, it does not^[182]. A recent study reported the effectiveness of fecal microbiota transplantation in a case of severe fistulizing CD with a sustained clinical remission for more than 9 mo after the treatment^[183]. Fecal microbiota transplants have also been used to manage *Clostridium difficile* infections in CD patients, and it appears to be effective in most of patients with a reduction or a complete resolution of the

infection-associated diarrhea^[182]. More clinical trials with better standardized protocols are required to confirm the beneficial effect of fecal microbiota transplantation in treatment of this complex disease.

CONCLUSION

Since the first description of CD in 1932, numerous research groups worldwide have attempted to unravel the complex and multifactorial etiology of the disease to develop a curative therapy. In addition to the identification of genetic and environmental risk factors in CD, increasing lines of evidence have supported a role for infectious agents in CD etiopathogenesis. These include the disruption of the intestinal barrier function associated with excessive bacterial translocation, an intestinal dysbiosis, defects in the secretion of IgA entrapping antigens and bacteria in the intestinal lumen and inefficacy of autophagy-mediated clearance of intracellular bacteria. These defects, which have been reported in CD patients, can lead to the emergence of infectious agents (viruses, eukaryotes or bacteria) that could induce chronic inflammatory characteristic of CD. Advances in the knowledge of infectious etiology of CD enable to develop different therapies based on the clearance of CD-associated pathogens, the modification of the imbalanced intestinal microbiota and re-establishment of a "healthy" microbiota with the use of antibiotics, probiotics and fecal microbiota transplantation. However, these therapies on their own are insufficient to provide a cure for CD. Therefore, successful CD therapies are likely to require multiple pathway-integrated treatments depending on the stage of the disease and each patient subset.

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Contribution of TLR signaling to the pathogenesis of colitis-associated cancer in inflammatory bowel disease

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Abstract

In the intestine a balance between proinflammatory and repair signals of the immune system is essential for the maintenance of intestinal homeostasis. The innate immunity ensures a primary host response to microbial invasion, which induces an inflammatory process to localize the infection and prevent systemic dissemination of pathogens. The key elements of this process are the germline encoded pattern recognition receptors including Toll-like receptors (TLRs). If pathogens cannot be eliminated, they may elicit chronic inflammation, which may be partly mediated *via* TLRs. Additionally, chronic inflammation has long been suggested to trigger tissue tumorous transformation. Inflammation, the seventh hallmark of cancer, may affect all phases of tumor development, and evade the immune system. Inflammation acts as a cellular stressor and may trigger DNA damage or genetic instability. Furthermore, chronic inflammation can provoke genetic mutations and epigenetic mechanisms that promote malignant cell transformation. Colorectal cancers in inflammatory bowel disease patients are considered typical examples of inflammation-related cancers. Although data regarding

the role of TLRs in the pathomechanism of cancer-associated colitis are rather conflicting, functionally these molecules can be classified as "largely antitumorigenic" and "largely pro-tumorigenic" with the caveat that the underlying signaling pathways are mainly context (*i.e.*, organ-, tissue-, cell-) and ligand-dependent.

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Key words: Inflammation; Tissue repair; Immunoregulation; Colitis-associated cancer; Toll-like receptor; Inflammatory bowel disease; Carcinogenesis

Core tip: Colorectal cancers arising in inflammatory bowel disease patients are considered typical examples of inflammation-associated cancers. The exact role of Toll-like receptor (TLR)-signaling in colitis-associated cancer initiation and development is conflicting. Here we aimed to summarize recent data on the contribution of TLR-mediated immune responses to inflammation-related colonic carcinogenesis.

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INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC), the main clinical phenotypes of idiopathic, relapsing-remitting inflammatory bowel disease (IBD) are systemic disorders affecting the GI-tract with frequent extraintestinal manifestations and other associated autoimmune conditions. IBD is considered a polygenic autoimmune disorder with a complex multifactor etiology. Generally, IBD arises in

susceptible individuals in whom upon an environmental trigger a sustained disturbed, deleterious mucosal immune reaction is provoked towards commensal microbiota^[1].

A balance between proinflammatory and repair signals of the immune system is essential for the maintenance of intestinal homeostasis. The interplay of genes regulating immune functions is strongly affected by the environment, especially gut resident microbiota. On the basis of genetic alterations in CD, impaired sensing and handling of intracellular bacteria by the innate immunity seem to be one of the most relevant pathophysiologic features^[1].

The innate immunity ensures a primary host response to microbial invasion, which induces an inflammatory process to localize the infection and prevent systemic dissemination of pathogens. The key elements of this process are the germline encoded pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), NOD-like receptors (NLRs), ribonucleic-acid (RNA) helicases, C-type lectin receptors, and cytosolic deoxyribonucleic-acid (DNA) sensors, which sense evolutionarily conserved pathogen-associated molecular patterns (PAMPs) of microbiota. The detection of PAMPs by PRRs triggers sequential activation of intracellular signaling pathways resulting in induction of a wide range of cytokines and chemokines that unite the early host response to infection^[2]. If pathogens cannot be eliminated, they may elicit chronic inflammation, which may be partly mediated *via* TLRs. Additionally, chronic inflammation has long been suggested to trigger tissue tumorous transformation. Indeed, a higher incidence of intestinal cancers has been observed in IBD patients. However, the exact role of TLR-signaling in colitis-associated cancer (CAC) initiation and development is still unknown, therefore, we aimed to summarize the currently available information on the contribution of TLR-mediated immune responses to inflammation-related colonic carcinogenesis.

RELATION OF TOLL-LIKE RECEPTORS TO COLONIC MUCOSA

The highly conserved TLRs represent sentinels of the innate immune system. TLRs belong to the type 1 transmembrane glycoproteins, which contain extracellular leucine-rich repeated sequences and Toll/interleukin-1 receptor (TIR) signaling domains. TLRs have five TIR-containing adaptor proteins, Myeloid differentiation factor 88 (MyD88), MyD88 adaptor-like (TIRAP), TIR domain containing adaptor inducing interferon- β (TRIF), TRIF-related adaptor molecule (TRAM)^[3], and sterile α and heat-armadillo motifs^[4]. TLR4 was the first receptor to be identified, and currently 10 TLRs have been identified in humans, and 13 in mice^[5]. TLRs are mainly expressed in the cells of the innate and adaptive immune system [*i.e.*, monocytes, macrophages, lymphocytes, mast cells, dendritic cells (DCs)], however, all TLR1-9 have also been identified as being expressed in human intesti-

nal epithelial cells (IECs)^[6-8].

TLRs usually recognize microbial wall components, as well as DNA and RNA fragments. TLRs bind specific motifs appearing in bacteria, fungi, protozoa, and viruses^[9,10]. These motifs are mainly lipids and lipopeptides (TLR-1, -2, -4, -6), bacterial flagellin (TLR5), and nucleic acid fragments (TLR-3, -7, -8, -9). TLR3 binds double-stranded RNA from viruses, while TLR7 and -8 can recognize single-stranded RNAs. Moreover, TLR7 recognizes immunoglobulin/self-RNA complexes within autoimmune disease conditions. Imiquimod is a specific ligand for TLR7. TLR9 is activated by bacterial and viral DNA, immunoglobulin-DNA complexes, and synthetic oligodeoxynucleotides (ODNs), which contain unmethylated CpG sequences^[9,10].

In vitro data have demonstrated hyporesponsiveness of IECs to TLR ligands^[7,8]. Antigen-presenting cells (APCs) in the lamina propria (LP) also seem to be unresponsive to TLR ligands^[11]. Under physiologic conditions, TLR3, -7, -8, and -9 are expressed in endosomes, or basolateral membrane (TLR5), where these TLRs are not exposed to pathogens unless microbiota get into the cells or invade mucosa^[2]. Apical epithelial TLR9 activation by bacterial DNA fragments has been reported to take part in colonic homeostasis^[12]. These findings underline a unique feature of TLRs (and other PRRs) in IECs that establishes immune tolerance to the commensal flora of the colonic mucosal interface.

In addition, epithelial TLRs contribute to balancing the composition of luminal microorganisms by regulating the secretion of different antimicrobial peptides and mucosal IgA. TLR9^{-/-} mice have impaired expression of cryptidin (α -defensin) compared to wild-type mice^[12]. Signaling through TLR-2, -3, and -4 have all been implicated with the expression of β -defensins in IECs^[13,14]. Several TLR signals in IECs induce B cell-activating factors leading to immunoglobulin class switch recombination in B cells of the LP without T cell activation, resulting in IgA secretion^[15]. Moreover, activation of TLR-3 and -4 has been found to induce epithelial expression of an epithelial immunoglobulin transporter (polymeric immunoglobulin receptor) that enhances luminal IgA secretion^[16,17].

To date, TLR signaling can be classified into classical/canonical and alternative/noncanonical pathways^[18]. All TLRs, except TLR3, utilize the MyD88-dependent signaling pathway to induce the expression of proinflammatory cytokine genes^[19]. TLR3 exclusively uses the TRIF pathway^[19]. The classical inflammatory signaling pathway is mainly activated through MyD88, which, in turn, recruits IRAKs and TRAF6^[20]. TRAF6 activates transforming growth factor- β activated kinase 1 which phosphorylates and activates the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase complex, finally resulting in the release and translocation of NF- κ B into the nucleus, thereby inducing the production of tumor necrosis factor (TNF)- α , interleukin (IL)-1, and IL-6, the key mediators of (intestinal) proinflammatory responses^[21-23]. However, TLR3 and some of the TLR4 signals utilize

the TRIF adaptor molecule signaling independently of MyD88. This alternative pathway culminates in the activation of TRAF3 and IRF3, resulting in the secretion of type I interferons (IFNs), even in the gut^[24]. TLR4 is unique among the TLRs as it can activate two distinct signaling pathways: the classical pathway (through TIRAP and MyD88) and the alternative pathway (*via* TRIF and TRAM)^[18].

ASPECTS OF COLITIS-ASSOCIATED CANCER

As early as ancient times, Hippocrates and Galenus realized the similarity between inflammation and cancer, and hypothesized that cancer evolved from inflammatory lesions^[25]. In 1863, Rudolf Virchow observed a close etiologic relation between chronic inflammation and carcinogenesis, realizing that tumors possess a typical “lymphoreticular infiltrate”^[26,27]. The first evidence of the antitumoral effects of microbial products dates to the beginning of the 18th century, when Deider reported that infection in patients with cancer could be accompanied by the remission of malignancies^[28]. In the 1890s, William B. Coley, a surgeon from New York, observed that repeated injections of a mixture of bacterial toxins served as an efficient antitumoral therapeutic agent^[29]. Later, in 1943, lipopolysaccharide (LPS) was discovered as the “hemorrhage-producing fraction” of Coley’s lysate, which accounted for its antitumoral effects^[28]. After the discovery of TLRs, their ligands and signaling pathways, it was found that microbe-derived factors act by stimulating TLR signaling and activating both the innate and adaptive immune responses to enhance anti-tumor immunity^[30].

In 2000, Hanahan and Weinberg^[31] proposed a model to define the six hallmarks of carcinogenesis. Generally, inflammation is required to fight microbial infections, heal wounds, and maintain tissue homeostasis, however, it can lead to cancer. Inflammation, the seventh hallmark of cancer may affect all phases of tumor development, including tumor initiation, promotion, invasion and metastatic dissemination, and can evade the immune system. Inflammation acts as a cellular stressor and may trigger DNA damage or genetic instability. Furthermore, chronic inflammation can provoke genetic mutations and epigenetic mechanisms which promote malignant cell transformation. Based on these results, nowadays increasing evidence suggests that inflammation should also be included in this list^[18,32]. In inflammation, a peculiar tissue microenvironment is induced with the capacity to tolerate tumor cell growth and metastasis by altering the immunoregulatory mechanism, and thus making the immune system incapable of destroying tumor cells^[18]. Moreover, the expression of TLRs in tumor cells may directly or indirectly contribute to tumorigenesis in several tissues and organs. Activation of TLR signaling pathways may promote tumor invasion, apoptosis resistance, chemoresistance, tumor progression and metastasis development^[18].

In chronic inflammatory conditions, when organs with large epithelial surfaces are affected, as in IBD, the epithelial barrier function is critical for disease onset. As the epithelium is densely inhabited by resident microbial flora, the role of native immunity is particularly important in recognising and distinguishing commensal enteric bacteria from invading bacteria, and thus, in maintaining tolerance and homeostasis. Subsequently, the chronic unrestrained inflammatory response which occurs in IBD is mainly driven by a disintegrated host immune regulatory network, and is further responsible for the increased susceptibility to colorectal cancer (CRC).

TLRs are involved in the maintenance and functioning of the epithelial barrier integrity in the gut and regulating the MyD88 adaptor protein. Therefore, TLRs may display a protective function in the control of intestinal inflammation and inflammation-associated cancer^[33]. Colorectal cancers in IBD patients are considered typical examples of inflammation-related cancers. However, tumors usually appear after several years of active disease, with a cumulative lifetime risk of 18%-20% in UC, and up to 8% in CD^[34-36]. Indeed, recent epidemiological data indicate that over 25% of all cancers are related to chronic infection and other unresolved inflammation^[37]. Current results indicate that TLRs have a potential role in microbiota-associated gastrointestinal cancer metastasis through the recognition of microbiota ligands, initiating inflammation, and promoting tumorigenesis^[38].

Most colorectal cancers are sporadic without any obvious connection to intestinal inflammation. Interestingly, IBD patients also have an increased susceptibility to other malignancies, such as lymphomas/leukemias and hepatocellular carcinoma suggesting that local inflammation could not only have intestinal, but also systemic tumor-promoting effects, or the genetic alterations that affect inflammatory and immune homeostasis in IBD also predispose patients to cancer in other tissues^[39,40]. In IBD, the increased susceptibility to extraintestinal tumors could also be related to immunosuppressive treatment. However, the types of tumors increasingly found in IBD patients are different from those observed in transplant patients under immunosuppression^[41,42].

Both intrinsic and extrinsic inflammatory pathways are linked to carcinogenesis. Intrinsic inflammation is mainly initiated by mutations leading to oncogene activation as well as to inactivation of tumor suppressors. The extrinsic pathway in terms of infection or inflammation increases cancer risk. Although in IBD patients inflamed intestinal cells already have CRC-related genetic abnormalities before developing dysplasia, in CAC, genetic alterations seem only to be a secondary cause rather than a primary cause of carcinogenesis^[43]. It is likely that abnormalities in PRR signaling lead to dysregulated expression of genes and enzymes involved in cell proliferation, apoptosis, and DNA repair prior to gene alterations. Frequent alternative cycles of mucosal injury and repair in the presence of tumorigenic cytokines, chemokines, and prostaglandins may also predispose to genetic mutations,

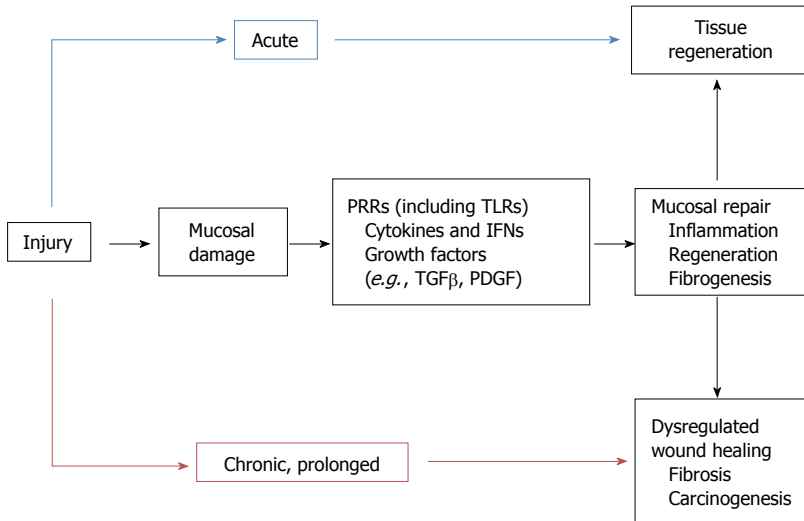


Figure 1 Relationship between pattern recognition receptors and tissue regeneration. In case of acute tissue injury, TLRs acts as mucosal healing factors. In the case of chronic, sustained inflammation, TLRs may promote dysregulated wound healing leading to cancer development. PRRs: Pattern recognition receptors; TLRs: Toll-like receptors; IFNs: Interferons; TGF: Transforming growth factor; PDGF: Platelet-derived growth factor.

which increase cancer risk^[44,45].

The definite similarities between tumor stroma and chronic wounds have led to the suggestion that cancers are wounds that do not heal, leading finally to uncontrolled tissue repair processes^[46] (Figure 1). CRC arises from the intestinal epithelium, a highly proliferative tissue which renews itself every several days under steady-state conditions. Repeated or prolonged wound repair responses to tissue injury may provoke malignant cell transformation^[47]. Epithelial regeneration and myofibroblast activation, two major events in wound-healing, are strongly influenced by TLR signaling. The contribution of TLR signals to regeneration can be found in the intestine, where a TLR2/TLR4/MyD88 cascade mediates mucosal healing in the regenerative phase of DSS-colitis^[48,49]. It has also been reported that TLR-mediated MyD88 signaling in macrophages of the LP regulate crypt stem cell differentiation and epithelial proliferation through cyclooxygenase-2 and prostaglandin (PG)E2 expression^[49,50]. TLR4 activation has also been shown to induce IEC proliferation *via* induction of EGFR ligands^[51,52]. Moreover, in inflammatory circumstances the surface expression of TLR-2 and -4 may be enhanced leading to IECs responsiveness to their ligands^[53,54]. Based on these results, it seems that abnormal TLR signaling may induce enhanced epithelial proliferation, and thus may contribute to colitis-associated carcinogenesis.

TOLL-LIKE RECEPTORS IN COLITIS-ASSOCIATED CANCER

Previous studies have shown that certain TLRs are expressed in colon cancers and colon cancer cell lines^[18]. In colorectal cancers, TLR3, -4, -5, -7, and -8 have been found to be expressed^[18], while several TLRs (including TLR7-9) are also expressed in the human colon carcino-

ma cell lines, HCT15, SW620 or HT29^[55-57]. TLR expression in tumor cells appears to promote tumorigenesis by facilitating survival and migration in a tumorous microenvironment that is characterized by chronic inflammation and PAMPs^[58]. On the other hand, the complicated interactions among tumor cells, immune cells, and PAMPs/DAMPs in the tumorous microenvironment may support an inappropriate immune response or antitumor immune tolerance through TLR signaling. With regard to tumorigenesis, a typical dual role of TLR signaling pathways has been proposed, as they may be critical for cancer cell survival and progression, however they may also elicit tumor death signaling. TLR-mediated signaling is directed toward cytoprotection or tumor cell suppression, thus the pro-survival or pro-death function is context-dependent, and influenced by many intra- and extracellular factors, such as the involved tissues, surrounding microenvironment, genetic background, and stage of tumor development, nevertheless its precise relation to cancer networks has not yet been fully elucidated^[18].

Toll-like receptor-mediated anti-tumorigenic effects

During the past two decades, studies have established that boosting TLRs and downstream mediators such as type I IFNs, can be used therapeutically to shift the balance from immunotolerance to antitumoral effects^[59].

The antigen-presenting capacity of tumor cells is poor, therefore, antitumoral immune responses usually depend on professional APCs like DCs^[60]. DCs have been a focus of cancer research due to their ability to initiate potent antitumoral immune responses. A lack of DC activation, often resulting from inhibitory signals from cancer cells, may also induce immune tolerance *via* T cell deletion or regulatory T cells (Tregs)^[60], which favors tumor progression. TLR-activated DCs can mediate antitumoral responses through antigen presentation, T cell activation, and direct cytotoxic effects on tumor cells^[61,62].

TLR5 activation on DCs as well as TLR9-stimulated plasmacytoid DCs promote antitumoral immunity^[63,64]. It is hypothesized that DC-mediated tumor cell killing triggers a more efficient antigen presentation to cytotoxic T cells, thus amplifying antitumoral responses.

Activation of TLRs on DCs regulates T cell activation not only *via* the class II major histocompatibility complex and co-stimulatory molecules, but also through TLR-induced signals in DCs that block the suppressive effect of regulatory T cells in an IL-6-dependent manner^[65]. Moreover, TLR8 activation can directly inhibit Treg function, hence support antitumoral immunity^[66]. TLR9 agonists as well as TLR-induced IFN α also have an important role since both are known to reduce tumor growth by blocking angiogenesis^[67,68].

In a recent study, the colonic tumor development modulatory effect of TLR5-dependent signaling was assayed in a mouse xenograft model of human colon cancer^[69]. The lack of MyD88 or TLR5 expression was found to enhance tumor growth and inhibit tumor necrosis. In contrast, TLR5 activation by peritumoral flagellin treatment substantially increased tumor necrosis, leading to significant tumor regression.

Within the TLR family, TLR9 is specifically stimulated upon sequence- and methylation-dependent DNA signaling. Self-DNA and oligonucleotides containing unmethylated CpG motifs are also sensed by and activate TLR9. Modifications in the structure of nucleic acids influence their immunomodulatory, *i.e.*, agonistic or suppressive, as well as pro- or anti-tumorigenic capacity^[57,70]. TLR9 activation by synthetic oligodeoxynucleotide agonists (CpG-ODN) has also demonstrated antitumor activity in xenograft models of murine colon cancer^[71]. Moreover, TLR9 agonists induce type I IFN secretion in DCs finally resulting in cytotoxic DCs, activated NK cells and cytotoxic T cells, all of which possess a remarkable antitumor immune response^[72,73].

Toll-like receptor-mediated pro-tumorigenic effects

TLRs may act as tumor promoting factors by transmitting proinflammatory, anti-apoptotic, proliferative or pro-fibrogenic signals in either the tumor cells or the tumorous microenvironment.

TLRs are key elements of inflammatory signaling which can be mediated by MyD88-dependent and MyD88-independent pathways. Enhancement of the signaling pathway of transcription factor nuclear factor (NF)- κ B is one of the major tumor-promoting effects of TLRs. TLR activation upregulates several tumorigenic inflammatory cytokines (*e.g.*, IL-1 β , TNF α , IL-6) in a NF- κ B-dependent manner^[59,74,75], which transcriptionally controls a large set of target genes that play important roles in cell survival, inflammation, and immune responses^[76].

TLR signaling is also involved in the inhibition of apoptosis. NF- κ B is considered an important anti-apoptotic pathway controlling the expression of anti-apoptotic genes and restricting the activation of pro-apoptotic pathways^[77,78]. TLR signaling can activate NF-

κ B through MyD88-dependent and MyD88-independent pathways, moreover, the TLR-mediated release of IL-1 β and TNF α promotes NF- κ B activation. In colorectal cancer, TLR-induced NF- κ B activation has been found to facilitate tumor cell survival^[48]. Furthermore, in the MC26 mouse colon cancer cell line TLR4 activation was found to mediate resistance of tumor cells to cytotoxic T cell-mediated cell death and favor tumor growth^[55].

The TLR-mediated promotion of wound healing may also lead to cancer development. After DSS-mediated injury, TLR2 and TLR4 activation facilitates epithelial repair *via* the MyD88-dependent pathway^[48], and TLR-MyD88 signaling also regulates the expression of epiregulin, which may contribute to colon cancer development^[78].

In mice, Faubion *et al.*^[79] demonstrated that chronic inflammation arising from the bowel may induce thymic involution and Treg cell suppression. These events are suggested to lead to the enhancement of inflammation-mediated processes, and worsen IBD. Restoration of homeostasis through suppression of TNF α production and fortification of Treg cells were proposed for the treatment of human IBD^[80]. In the existing connection between TLR-signaling and Treg cells^[65,81], these data on IBD may support the concept that uncontrolled inflammation weakens the Treg-mediated inhibition and increases the risk for inflammation-associated carcinogenesis.

Controversial data exist regarding the role of TLR2 in CAC. In one study, increased tumor development and higher IL-6, IL-17A and phospho-STAT3 levels were reported in a TLR2-deficient azoxymethane (AOM)-dextran sodium sulfate (DSS) murine model^[82], while there were no differences in CAC between wild-type and TLR2-deficient AOM-DSS colitic animals^[83].

The pro-tumorigenic role of TLR4 in CAC is well established. The intestinal microbiota, which normally colonize mucosal surfaces in symbiotic mutualism with the host is unique and quite stable over time^[84]. The basic challenge for intestinal immune recognition is the requirement of a simultaneous delicate balance between tolerance and responsiveness towards microbes^[85]. Several data suggest the existence of immune tolerance to antigens in an individual's bacterial flora, whereas its breakdown definitely contributes to IBD pathogenesis^[86]. In the colon, where there is a constant interaction between microbiota and IECs, TLR4 deletion significantly reduces inflammation and tumor size in the AOM-DSS induced CAC-model in mice^[87]. Additionally, overexpression of the constitutively activated TLR4 exhibits a higher sensitivity to CAC in a transgenic mouse model^[88]. Other studies^[89,90] also support the results that both the deletion of the TLR4 adaptor MyD88 molecule and the depletion of TLR4 activating gut microbiome reduce colon cancer development.

CONCLUSION

Inflammation affects many aspects of tumorigenesis. One of the important discoveries in the field of TLR sig-

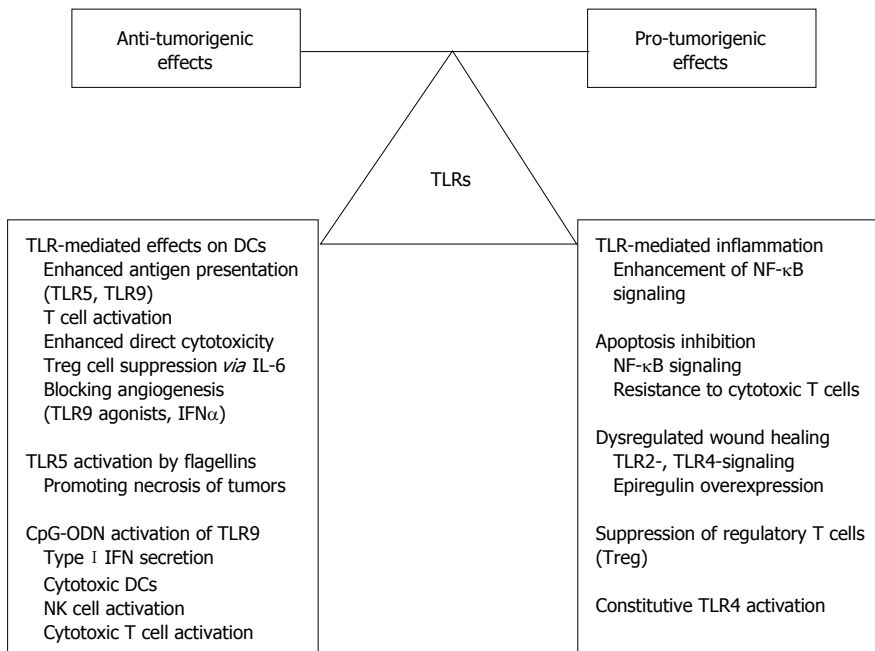


Figure 2 Dual role of Toll-like receptor signaling in colitis-associated carcinogenesis. While some direct and indirect effects of TLR-signaling act largely as an anti-tumorigenic factors, other effects may promote cancer development. TLR: Toll-like receptor; DC: Dendritic cell; IL: Interleukin; IFN: Interferon; ODN: Oligodeoxynucleotide; NF- κ B: Nuclear factor- κ B.

naling and inflammation-associated cancer biology is the realization that TLRs may promote or suppress tumor formation in certain organs, including the intestine.

Although recent data regarding the role of TLRs in the pathomechanism of CAC are rather conflicting, functionally they can be classified as “largely antitumoral” and “largely tumorigenic” molecules with the caveat that the underlying signaling pathways are mainly context, (*i.e.*, organ-, tissue-, cell-) and ligand-dependent (Figure 2). Advanced exploration and better understanding of the relationship amongst TLR-signaling, inflammatory microenvironment and colitis-associated carcinogenesis should provide further insight into cancer development in IBD patients.

Further detailed functional analyses of TLRs in inflammation-related tumor biology are needed to explore and define more precisely the subcellular and molecular mechanisms, hopefully allowing the introduction of selective new therapeutic approaches into daily practice.

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Capsule endoscopy in Crohn's disease: Are we seeing any better?

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Core tip: Crohn's disease (CD) is a complex, immune-mediated disorder that often requires a multi-modality approach for optimal diagnosis and management. Over the past decade, capsule endoscopy (CE) has increasingly found a place in the algorithm for the diagnosis, treatment and monitoring of CD. CE potentially offers a noninvasive approach to evaluate areas of the small bowel that may be difficult to access with traditional endoscopy. Furthermore, CE has potential application for specific subsegments of patients with inflammatory bowel disease (IBD), such as those with IBD unclassified, pediatric patients and patients with CD who have previously undergone surgery.

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Abstract

Crohn's disease (CD) is a complex, immune-mediated disorder that often requires a multi-modality approach for optimal diagnosis and management. While traditional methods include ileocolonoscopy and radiologic modalities, increasingly, capsule endoscopy (CE) has been incorporated into the algorithm for both the diagnosis and monitoring of CD. Multiple studies have examined the utility of this emerging technology in the management of CD, and have compared it to other available modalities. CE offers a noninvasive approach to evaluate areas of the small bowel that are difficult to reach with traditional endoscopy. Furthermore, CE maybe favored in specific sub segments of patients with inflammatory bowel disease (IBD), such as those with IBD unclassified (IBD-U), pediatric patients and patients with CD who have previously undergone surgery.

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INTRODUCTION

Crohn's disease (CD) is a complex, immune-mediated condition. Diagnosis and management requires information from multiple modalities. These include clinical symptomatology, serologic and fecal testing, endoscopic assessment with histopathologic analysis and radiologic imaging. While the majority of CD patients have involvement of the small bowel, up to 30% have disease that is confined to the small-bowel alone^[1]. Historically, the small bowel was assessed using small bowel radiography, ileocolonoscopy, or push enteroscopy. With advances in technology and imaging, there are now other options

for evaluating the small bowel including computed tomographic enterography (CTE), magnetic resonance enterography (MRE), contrast ultrasonography, double balloon enteroscopy and capsule endoscopy (CE). CE offers a sensitive and noninvasive strategy for establishing the correct diagnosis, and the monitoring of disease activity. Findings on CE in CD include aphthae, deep ulcerations, and stricturing disease (Figure 1). Furthermore, CE is particularly useful in areas of the gastrointestinal tract that are not optimally seen on conventional endoscopy or radiologic imaging.

CE (Given, Yoqneam, Israel; Pill-Cam SB) was Food and Drug Administration (FDA) approved in 2001 for evaluation of the small bowel. CE is currently approved for the evaluation of obscure gastrointestinal bleeding and iron deficiency anemia in patients with a normal upper and lower endoscopy, as well as for the evaluation and monitoring of CD. Given imaging has recently launched its third generation of small bowel capsules, PillCam SB3, which has improved image detail, and adaptive frame rate technology leading to increased visualization of the small bowel, and improved efficiency. A competing capsule system was developed by Olympus (Lake Success, NY; EndoCapsule) that was FDA approved in 2007. Although the technology continues to improve, the main barrier to CE use in IBD has been its lack of specificity and concern for retention in the small bowel due to strictures^[2-4].

ESTABLISHING A DIAGNOSIS OF CD

Clinicians have relied upon endoscopic and histologic evaluation coupled with small bowel imaging, to define the location and extent of involvement in CD. Improvements in imaging technology have now led to the widespread use of cross sectional imaging in the diagnosis of CD (Table 1).

A number of studies have compared radiologic imaging studies to CE in CD. Albert *et al*^[5] prospectively compared CE to magnetic resonance imaging (MRI) and small bowel enteroclysis (SBEC) in 52 known or suspected CD patients. Of the 52 patients included in the study, 41 were confirmed to have CD. Following radiologic testing, 14 patients were noted to have strictures, with only the 27 remaining patients undergoing CE. CE detected small bowel lesions in 93% of patients, as compared to 78% and 33% for MRI and SBEC, respectively. Although the absolute difference in sensitivity favored CE, the study was underpowered to achieve statistical significance.

Hara *et al*^[6] prospectively imaged the small bowel of 17 patients with known or suspected CD with CE, CTE, colonoscopy with ileoscopy, and small bowel follow through (SBFT). The mean time between the first and last exam was 20 wk. The diagnostic yield, defined as the number of patients with evidence of CD over the number of patients studied, was 71% with CE, 65% with ileoscopy, 53% with CTE, and 24% with SBFT. Due to the small sample size, the study did not reach statistical

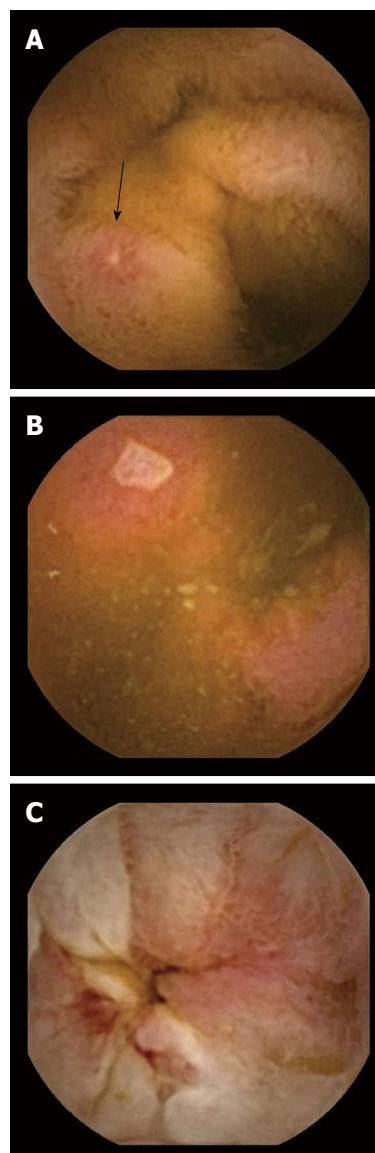


Figure 1 Findings on capsule endoscopy in Crohn's disease. A: Aphthous ulceration; B: Shallow ulcer; C: Small bowel stricture.

significance. This study was further limited by a lack of specificity as any erosion or ulcer seen on capsule endoscopy or ileoscopy was classified as CD. A major limitation of small bowel imaging is the lack of a reference standard for the diagnosis of CD. Without a gold standard, a sensitivity or specificity of CE in diagnosing small bowel CD cannot be calculated.

Solem *et al*^[7] conducted the only prospective study to overcome this limitation directly comparing CE to CTE, ileocolonoscopy, and SBFT using a consensus diagnosis based on clinical presentation, laboratory data, and the four imaging studies. Each interpreter classified patients with either active, suspicious, inactive or absent CD. These studies were performed sequentially over 4 d with CTE as the first exam. If no strictures were seen on radiologic imaging then the patient underwent CE followed by SBFT. Forty-two patients enrolled in the study, but only 28 underwent CE secondary to stricture, abscess, or

Table 1 Studies evaluating diagnostic yield of capsule endoscopy

Ref.	Compared modality	n	Diagnostic yield of VCE	Incremental yield of VCE	P value
Albert <i>et al</i> ^[5]	MRE	27	93%	15%	NS
	SBEC			60%	
Hara <i>et al</i> ^[6]	CTE	17	71%	18%	NS
	Ileoscopy			6%	
	SBFT			47%	
Solem <i>et al</i> ^[7]	CTE	28	83%	1%	NS
	Ileocolonoscopy			9%	
	SBFT			18%	
Dionisio <i>et al</i> ^[9]	SBR	428	58%	37%	< 0.0001
	Ileocolonoscopy	236	64%	15%	0.000
	CTE	119	70%	39%	< 0.00001
	PE	102	50%	42%	< 0.00001
	MRE	123	50%	7%	NS
Jensen <i>et al</i> ^[10]	MRE	93	100%	27%	0.03
	CTE		100%	19%	NS

CE: Capsule endoscopy; MRE: Magnetic resonance enterography; CTE: Computed tomography enterography; SBEC: Small bowel enteroclysis; SBFT: Small bowel follow through; SBR: Small bowel radiography; PE: Push enteroscopy; NS: Non-significant.

drop out. The authors found that the sensitivity of CE for active CD did not differ significantly from the other three modalities. The sensitivities were 83% for CE, 82% for CTE, 74% for ileocolonoscopy, and 65% for SBFT. The specificity of CE, however, was 53%, which was significantly lower than all other modalities.

Using histopathology for diagnosis of CD, Dubcenco *et al*^[8] evaluated the accuracy of CE in known or suspected CD. Thirty-nine patients without strictures underwent ileocolonoscopy, small bowel series (36 with SBFT and 3 with small bowel enema), and CE within an average of 22 d. Histology was able to confirm disease in 25/39 patients, exclude disease in 10/39 patients, and in 4 patients the tissue was not accessible. The sensitivity and specificity for CE was 89.6% and 100% respectively, with the sensitivity and specificity for small bowel series was 27.6% and 100% respectively.

A meta-analysis was performed by Dionisio *et al*^[9] including 19 trials, comparing CE to small bowel radiography (SBR), ileocolonoscopy, CTE, push enteroscopy (PE), and MRE in nonstricturing CD. The primary outcome measure was weighted incremental yield (IY_w), defined as the diagnostic yield of capsule endoscopy minus the diagnostic yield of the comparative modality. For suspected CD, CE was superior to CTE with an IY_w of 47% (68% *vs* 21%), SBR with an IY_w of 32% (52% *vs* 16%), and ileocolonoscopy with an IY_w of 22% (47% *vs* 25%). For known CD, CE was superior to PE with an IY_w of 57% (66% *vs* 9%), SBR with an IY_w of 38% (71% *vs* 36%), and CTE with an IY_w of 32% (71% *vs* 39%). There was no benefit found of CE over MRE likely secondary to the small sample size in this meta-analysis.

Jensen *et al*^[10] evaluated the diagnostic accuracy of CE compared to CTE and MRE. This study was not included in the previously mentioned meta-analysis. Ninety-three patients with suspected or newly diagnosed CD were enrolled in the study. Twenty-one patients were diagnosed with terminal ileal CD based on the gold standard of histopathology from ileocolonoscopy and/or surgery. Based

on the 21 patients with ileal CD, the sensitivity of CE compared to CTE was 100% *vs* 76%, respectively ($P = 0.03$). The sensitivity of CE was higher than MRE, 100% *vs* 81%, respectively; however this was not statistically significant. Specificities and positive predictive values were comparable across the three modalities.

The pediatric population with CD poses another area where the use of CE may prove beneficial. The FDA initially approved CE for pediatric use between the ages of 10 and 18 years of age in 2003, then expanded this age range in 2009 for children as young as 2 years of age^[11]. Physiologic differences exist between children and adults that change the risks associated with sedation in the pediatric population, and may result in a higher threshold to perform an invasive endoscopy in children. CE was therefore studied as an alternative to traditional endoscopy in pediatric patients with suspected CD. In one study, MRE was compared to capsule endoscopy in a pediatric population with suspected CD^[12]. Ileocolonoscopy was the gold standard, and 19/60 patients included in this study were diagnosed with CD. The sensitivity and specificity of CE and MRE were comparable, 91%, 92% and 100%, 97.6%, respectively.

A study by Levesque *et al*^[13] investigated whether CE was a cost effective tool in evaluating small bowel CD in patients with two previous negative tests using a decision analytic model. In this model, CE was not a cost-effective strategy after a negative ileocolonoscopy and a negative radiologic study (either CTE or SBFT) with a QALY of \$500000. The high cost of capsule endoscopy is directly related to the poor specificity of the test, and from a lack of standardization in grading and diagnosing CD. A limitation of this study was the model did not take into account the extent or severity of the disease, or the costs associated with changes in management, or lack thereof.

Based on the most recent evidence based recommendations^[14], patients with a high suspicion for CD with a negative ileocolonoscopy should next undergo small bowel imaging. If the patient has signs or symptoms of a

Table 2 Capsule endoscopy in inflammatory bowel disease unclassified *n* (%)

Ref.	<i>n</i>	Results
Mow <i>et al</i> ^[2]	22	9 (40) suspected CD; 5 (23) confirmed CD
Maunoury <i>et al</i> ^[19]	30	5 (17), CD
Mehdizadeh <i>et al</i> ^[20]	21	6 (29), CD
Murrell <i>et al</i> ^[21]	68	15 (22), SB findings

CD: Crohn's disease; SB: Small bowel.

stricture and/or obstruction the modality of choice is either CTE, MRE, or patency capsule. If there are no signs or symptoms of obstruction, CE is a good option due to its high sensitivity.

USE OF CE IN IBD UNCLASSIFIED

In approximately 15% of patients with isolated colitis it is difficult to definitively distinguish between CD and ulcerative colitis (UC)^[15,16]. These patient phenotypes were originally called indeterminate colitis (IC)^[17], and are now classified as IBDU. The clinical course and prognosis of IBDU may be worse than UC, especially in patients that have undergone an ileal pouch anal anastomosis (IPAA) (Table 2)^[18].

Maunoury *et al*^[19] evaluated the role of CE in 30 patients with IBDU and negative ASCA and pANCA. As in the paper cited above, the definition of suspected CD that was used was the presence of three or more small bowel ulcerations, however this definition has not been prospectively validated. Five patients had findings suspicious of CD. In long term follow up, another five patients with normal CE were diagnosed with CD on repeat ileocolonoscopy.

Mehdizadeh *et al*^[20] evaluated the utility of CE in ulcerative colitis (UC) and IBDU. The indications for CE in UC patients were atypical symptoms, disease refractory to medical therapy, and new onset symptoms after total proctocolectomy with IPAA. Nineteen of the 120 patients (16%) had findings consistent with CD, defined as three or more small bowel ulcerations. In addition, 6 of the 21 patients (29%) who had undergone surgical treatment for UC (IPAA) were found to have CD.

Murrell *et al*^[21] investigated the role of preoperative CE in predicting outcomes after IPAA. This retrospective study identified 68 patients, 48 with UC and 20 with IBDU, who had a CE prior to surgery. CE was positive in 15 patients defined as ulcerations, erosions, or erythema. There was no correlation found between positive CE findings and acute pouchitis, chronic pouchitis, or *de novo* CD over a median follow up time of 12 mo.

We believe that all patients with IBDU should have some form of small bowel imaging to evaluate for CD, with capsule endoscopy as one of the options. Even if the decision to proceed with surgery is unchanged by the findings of a few nonspecific ulcerations in the small bowel, it may be useful to help set expectations for disease course subsequent to IPAA construction.

Table 3 Capsule endoscopy in postoperative Crohn's disease

Ref.	Compared modality	<i>n</i>	Results
Bourreille <i>et al</i> ^[26]	IC	31	IC Sens/Spec: 90%/100% CE Sens/Spec: 62%-76%/90%-100%
Pons Beltran <i>et al</i> ^[27]	IC	24	IC recurrence: 25% CE recurrence: 68%

IC: Ileocolonoscopy; CE: Capsule endoscopy; Sens/Spec: Sensitivity/Specificity; SICUS: Small intestine contrast ultrasonography.

USE OF CE FOR POSTOPERATIVE RECURRENCE OF CD

CD typically recurs after ileocolonic resection just proximal to the surgical anastomosis. Endoscopic recurrence rates range from 70%-90% at one year with clinical recurrence rates of about 30% at three years^[22,23]. The current recommendation for diagnosing recurrent CD is an ileocolonoscopy between 6 mo to 1 year after resection^[24,25]. Noninvasive techniques including CE have been studied to assess for postoperative recurrence of CD. Aside from the benefit of a noninvasive test, CE should theoretically improve visualization of the neoterminal ileum, as the altered postoperative anatomy often can complicate intubation and visualization of the neoterminal ileum with standard ileocolonoscopy (Table 3).

Bourreille *et al*^[26] prospectively compared ileocolonoscopy (gold standard) to CE in the postoperative setting in 32 patients at a median time of 6 mo after surgery. Two independent observers interpreted the CE results. One patient was excluded because his neoterminal ileum was not visualized with ileocolonoscopy. Ileocolonoscopy detected recurrence in 19 of the 21 patients with a sensitivity of 90% and a specificity of 100%. In comparison, the sensitivity and specificity of CE were 62%-76% and 90%-100%, respectively. Interestingly, CE found proximal small bowel lesions, out of reach of the ileocolonoscopy, in two thirds of the patients, however these findings are of uncertain clinical significance.

A similar study by Pons Beltran *et al*^[27] reached a different conclusion. Out of 19 evaluable patients, ileocolonoscopy detected recurrence in only 25% of the patients, while capsule endoscopy detected recurrence in 68% of the patients. As in the previous study 59% had proximal small bowel lesions found on CE. The low detection rates of ileocolonoscopy are partially explained by the postsurgical anatomy, where the neoterminal ileum could not be reached in 3 patients. All of the patients included had a side to side anastomosis which may limit visualization of the neoterminal ileum. When assessing for patient comfort, all patients preferred CE over ileocolonoscopy.

All of the mentioned studies have small samples sizes. We do not recommend replacing ileocolonoscopy with CE for evaluation for recurrent CD at a surgical anastomosis that is reachable *via* ileocolonoscopy. However, in patients where the neoterminal ileum cannot be intubated or in patients with symptoms or abnormal labs (elevated

Table 4 Influence of capsule endoscopy findings on management of Crohn's disease

Ref.	n	CE findings	Change in management
Dussault <i>et al</i> ^[28]	71	¹ Moderate 45%	54%
		² Severe 17%	75%
Long <i>et al</i> ^[29]	86 (with CD)	¹ Moderate 30.3%	51%
		² Severe 47.6%	73%
Min <i>et al</i> ^[30]	50 (with CD)	86%	75%

¹Moderate endoscopic lesions: erythema and a few aphthous ulcers; ²Severe endoscopic lesions: defined as multiple and/or deep ulcers and/or stenosis with/without retention. CD: Crohn's disease; CE: Capsule endoscopy.

C-reactive protein or anemia) with a negative ileocolonoscopy. CE may identify inflammation elsewhere in the small bowel, though we expect this to be a small group of patients.

INFLUENCE OF CE FINDINGS ON MANAGEMENT OF CD

There is limited data on how CE may change management in patients with established CD (Table 4).

In a retrospective cohort of 71 established CD patients, Dussault *et al*^[28] evaluated how CE impacts treatment decisions. These patients underwent CE for either unexplained anemia, discrepancy between clinical and endoscopic findings, disease assessment, or for evaluation of mucosal healing. Moderate endoscopic lesions, defined as erythema and a few aphthous ulcers, were found in 32/71 patients (45%). Severe endoscopic lesions, defined as multiple and/or deep ulcers and/or stenosis without retention, were found in 12/71 patients (17%). There was a change in medication in 38/71 (54%) patients three months after CE, of which 27 initiated a new medication most commonly an immunomodulator or an anti-tumor necrosis factor (TNF) agent. Two patients required surgery. Seventy-five percent of patients with severe endoscopic lesions had a change in their management, compared to 53% with moderate lesions, and 4% with normal CE.

Long *et al*^[29] performed a retrospective cohort study of 128 CE studies in established IBD patients between 2003 and 2009 at a tertiary referral center. Four capsule studies were excluded since they were retained in the stomach. Indications for CE included CD in 86, IBDU in 15, and pouchitis in 23 patients. The median duration of CD or IBDU was 6 years. The IPAA group had longer disease duration of 13.5 years. The majority of CD patients had either a few or multiple ulcerations with 22% having a normal CE. Sixty-two percent of the CD patients had a change in their medical management, defined as initiation or discontinuation of an IBD specific medication. Budesonide was the most common initiated medication with 40% of the CD patients starting a new medication. Eighty percent of the IBDU patients had a normal CE or mild erythema. Two thirds of these patients had a change in medical management with 40% initiating a new IBD specific medication, most commonly

prednisone or budesonide. In addition, 44% of the IPAA patients had a normal CE or mild erythema with 57% of them starting a new medication. We suspect that given the disconnect between CE and treatment changes, the clinical symptoms or biomarkers played a more important role in management decisions. Capsule retention occurred in 15 of the CD patients, leading to either a small bowel resection or strictureplasty in 13% of the CD patients.

Overall, in patients with minimal findings on CE, 51% had a change in medical management compared to 73% of patients with severe findings. Due to the retrospective nature of this study, it is unclear if management changes were due to the CE findings or other factors. It is also unknown whether these medications changes impacted the disease course. In addition, only a small number of patients initiated immunosuppressants or biologics.

The effect of CE on the management of CD has been studied in the pediatric population as well. Min *et al*^[30] evaluated whether CE will change management in the pediatric population. Indications for CE included active CD with poor growth in 50, IBDU in 16, and suspected IBD in 17 patients, respectively. Treatments and clinical outcomes were recorded before and one year after CE was performed. The overwhelming majority of patients with CD had abnormal CE findings (86%). Treatment escalation was required in 75% of patients, with the majority adding an anti-TNF agent, and 18% adding an immunomodulator. Follow up of these patients one year after capsule endoscopy showed statistically significant improvement in growth parameters, clinical indices, and laboratory markers. Given that the majority of patients requiring dose escalation had poor growth or active symptoms, it would appear that CE played a supportive role rather than a definitive role.

Flamant *et al*^[31] studied the prevalence and significance of jejunal lesions on CE in established CD patients. CE studies from 108 CD patients were analyzed retrospectively, and 56% of these patients were found to have jejunal lesions (17% isolated to the jejunum). On multivariate analysis, jejunal lesions were the only factor associated with an increased risk of disease relapse over a median of two years with an adjusted hazard ratio of 1.99 (95%CI: 1.10-3.61, $P = 0.02$). These data suggest that patients with proximal lesions may benefit from management top down strategy. This was demonstrated by Lazarev *et al*^[32] who identified jejunal disease being associated with multiple abdominal surgeries.

While it does appear important to identify patients with jejunal disease given their higher risk for a complicated disease course, it's not clear whether any particular imaging modality has the advantage.

USE OF CE FOR MUCOSAL HEALING

Data for the use of CE to monitor disease or evaluate treatment efficacy in CD is limited. A small case series from Athens prospectively investigated the correlation of

mucosal healing by CE with clinical response, defined as a CDAI drop of > 100 or a CDAI < 150 ^[33]. Diagnosis of CD was confirmed histologically in 34 of the 40 patients. Patients included had active symptoms and the initial CE was done prior to any treatment. After treatment initiation, patients were followed up regularly until there was clinical improvement. Within one of day symptom improvement a second CE was performed to evaluate for mucosal healing. Three endoscopic variables were used for mucosal healing; number of aphthous ulcers, number of large ulcers, and the period of time that any endoscopic lesion was visible. The number of large ulcers significantly decreased, while the number of aphthous ulcers remained unchanged. Because only one of the three markers improved, the authors concluded that clinical response did not correlate with mucosal healing. A major limitation of this study was the significant heterogeneity among treatments, which were chosen by the managing physicians. Only 6 patients received immunomodulators and steroids or anti-TNF agents, and they had the most significant endoscopic improvement.

In an abstract presented at the 2013 Crohn's and Colitis Foundation of America meeting, Shafran *et al*^[34] retrospectively evaluated whether mucosal healing defined by CE was critical in management decisions. Twenty-three patients were analyzed of which 17 achieved mucosal healing, determined by a single expert physician reader along with a Lewis score if available. Of the 17 patients with mucosal healing, 15 remained in clinical remission on their current treatment plan. In the 6 patients who did not achieve mucosal healing, four patients achieved clinical remission with changes in medications, 1 patient required surgery, and 1 patient entered a clinical trial.

Larger prospective trials are needed to confirm the utility of serial CEs to assess mucosal healing in patients with nonstricturing isolated small bowel CD. It will be important to identify these patients early as they may benefit from more intensive treatments, but it is unclear if CE would be superior to radiology.

CAPSULE RETENTION

Capsule retention is the greatest concern in patients with IBD. Adding to this concern is that the majority of patients do not visualize the capsule passing in the stool. In a systematic review involving 227 articles with 22840 capsule studies, the overall pooled retention rate was 1.4%. The pooled retention rate for established CD was 2.6%^[3].

Capsule retention does not necessarily require surgical intervention. Some patients with retained capsules are asymptomatic, while others can be managed medically with steroids or anti-TNF agents, to allow for passage through an inflammatory stricture. Other cases can be managed endoscopically with retrieval accomplished by deep enteroscopy or colonoscopy. Surgery with resection of the strictured segment or strictureplasty is sometimes required.

Although capsule retention should be avoided, some believe that retention leads to the appropriate diagnosis

and management by allowing for localization of the culprit lesion. In a study by Cheifetz *et al*^[35] CE was used in 19 patients with suspected small bowel obstruction based on symptoms or imaging. The capsule was retained in four patients proximal to the stricture and they all underwent elective surgery. Since the capsule was not lodged in the stricture, emergent surgery was not indicated in any of these patients. The operative findings of these four patients were deep ulceration and stricture in midjejunum, a jejunal anastomotic stricture, focal ulcerated stricture in the jejunum, and an ileal stricture.

Given imaging has developed the Agile patency capsule, which is the same size as Pillcam SB. The capsule is composed of lactose and barium with an impermeable membrane that disintegrates in less than 30 h. It contains a radiofrequency identification chip, which can be detected by a scanner if retained in the small bowel.

Herrerias *et al*^[36] evaluated the Agile patency capsule in 106 patients with known intestinal strictures. In this series, 13 patients had obstructive symptoms that were possibly or probably secondary to the Agile patency capsule. The Agile patency system is recommended when there is a suspicion for an intestinal stricture or obstruction to anticipate the subsequent safe passage of CE.

SCORING SYSTEMS FOR CAPSULE ENDOSCOPY

A limitation of CE and of many of the studies reviewed in this article is interobserver variability. Two main scoring systems have been developed to address this problem. The Lewis Score quantifies mucosal change and disease extent by assessing villous appearance, presence of ulcers and stenosis over each segment of small bowel^[37]. In 2008, Gal *et al*^[38] developed the capsule endoscopy crohn's disease activity index which evaluates inflammation, extent of disease, and presence of stricture, all graded on a numeric scale with the small bowel divided into proximal and distal halves. The Lewis score has been made more accessible as it was incorporated into the PillCam software (Given, Rapid Reader). Nevertheless, neither scoring system is utilized routinely in clinical practice. Furthermore, there is no definitive correlation between the score and the patient's clinical status or CDAI^[11]. Although scoring systems may be useful for longitudinal monitoring of CD, they have not been validated for this purpose.

OUR PRACTICE

Multiple variables need to be taken into account when deciding which imaging test is best to the study the small bowel in patients with established or suspected CD. The availability of CTE is institution dependant, and there are concerns about cumulative ionizing radiation exposure in CD patients^[39]. MRE avoids radiation and allows for extraluminal imaging. It is often our first test of choice in newly diagnosed CD patients. However, CE is performed

by gastroenterologists and seems to have higher sensitivity for mucosal lesions compared to cross sectional imaging.

We use CE in post-operative evaluation when the neoterminal ileum cannot be intubated or in patients with symptoms, anemia, or biomarker elevation in the setting of a normal colonoscopy. Clinically, we can confirm the data of Efthymiou *et al.*^[33], when colonic mucosal healing did not result in correction of iron deficiency anemia and CE confirmed ongoing active inflammation (aphthous ulcers) in the small bowel. CE may be the preferred option in pediatric patients with nonstricturing disease as it seems to result in management changes that are more significant than in the adult population. Increasingly mucosal healing has become the preferred endpoint of medical management and CE, along with biomarkers and cross sectional imaging, will play an important role in noninvasive disease monitoring of the small bowel.

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***Mycobacterium avium* subspecies *paratuberculosis* in the etiology of Crohn's disease, cause or epiphenomenon?**

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CD or ulcerative colitis and in healthy controls. On the basis of their studies, several authors support a close relationship between MAP and CD. Although increasing evidence of MAP detection in CD patients is unquestionable, a clear etiological link still needs to be proven.

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Key words: *Mycobacterium avium paratuberculosis*; Crohn's disease; Inflammatory bowel disease; Johne's disease; Mycobacterial protein tyrosine phosphatase

Core tip: The etiology of inflammatory bowel disease (IBD) is unknown. Some analogies between Crohn's disease (CD) and Johne's disease, a granulomatous chronic enteritis of cattle and sheep caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) have been identified. Several studies have tried to clarify if MAP has a role in the etiology of CD. However, the involvement of MAP in CD is still debatable. The present article provides a literature review of the evidence in favor and against the "MAP-hypothesis", the methods commonly adopted to detect MAP and the role of antimycobacterial therapy in treating IBD patients. In particular, new mechanistic findings seem to encourage the CD-MAP relationship.

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Abstract

The origin of inflammatory bowel disease is unknown. Attempts have been made to isolate a microorganism that could explain the onset of inflammation, but no pathological agent has ever been identified. Johne's disease is a granulomatous chronic enteritis of cattle and sheep caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and shows some analogies with Crohn's disease (CD). Several studies have tried to clarify if MAP has a role in the etiology of CD. The present article provides an overview of the evidence in favor and against the "MAP-hypothesis", analyzing the methods commonly adopted to detect MAP and the role of antimycobacterial therapy in patients with inflammatory bowel disease. Studies were identified through the electronic database, MEDLINE, and were selected based on their relevance to the objective of the review. The presence of MAP was investigated using multiple diagnostic methods for MAP detection and in different tissue samples from patients affected by

INTRODUCTION

Inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) represent a group of

chronic inflammatory alterations, whose etiology is still unknown and is notoriously multifactorial. According to the most accepted hypothesis, CD and UC are the result of an inappropriate and deregulated intestinal immune response against the normal bacterial flora. This is due to a complex interaction between environmental, genetic and immune factors. The above-mentioned factors are all necessary, but none of them, if singularly considered, is enough to explain the etiopathogenesis of IBD. We will focus our attention on analyzing the available data on this latter theory, mainly on the possible involvement of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in IBD.

On the basis of analogies between CD and some forms of infective enterocolitis, the hypothesis of a specific mycobacterial etiology has been proposed. In particular, some authors have studied the potential role of MAP in CD. MAP is the responsible agent in Johne's disease (JD), a chronic granulomatous enteritis affecting cattle and sheep, and could be implicated in the etiopathogenesis of disease in humans, according to clinical and histological similarities between the two diseases^[1,2]. The MAP hypothesis has aroused great scientific interest since the 1980s, following data reported by Chiodini *et al.*^[3,4]. There is now a renewed interest regarding the association between CD and MAP, due to the improvements in microbiological and genetic methods^[3,4]. However, published data on this issue are still contradictory. Two main hypotheses exist: MAP could represent the "primum movens" in causing CD or, alternatively, it could constitute an epiphenomenon of the disease, later colonizing the already inflamed mucosa in these patients^[5].

INFECTIVE THEORY

Many attempts have been made to isolate a microorganism that could act as the "primum movens" in the etiology of IBD (Table 1), however, to date no pathogen has been convincingly identified. Numerous bacterial pathogens such as *Listeria monocytogenes*^[6], *Pseudomonas maltophilia*^[7], *Mycobacterium kansasii*^[8], *Bacterioides fragilis*^[9], and *Chlamydia pneumoniae*^[10], and viral agents including the measles virus^[11,12] and *Cytomegalovirus*^[13] have been proposed as the cause of CD, but none have been accepted due to a lack of firm evidence.

Wakefield *et al.*^[12] proposed that CD is due to chronic infection of submucosal endothelium in the gut by the measles virus, inducing a granulomatous reaction and a microinfarction pathological process. This theory was supported by epidemiological studies which associated perinatal exposure to the measles virus^[14] and the attenuated measles vaccine^[15] with an increased risk of the development of CD. Because of the absence of an increased risk for CD in vaccinated patients^[16], the difficulty of detecting the virus in intestinal specimens^[17] and low antibody titers to measles in CD patients^[18], this hypothesis was rejected and measles has now been proposed as a possible cofactor rather than the primary causative agent of CD.

Table 1 The infective theory of inflammatory bowel disease etiology

Infectious agent	Ref.	Current position
Bacterial		
<i>Listeria monocytogenes</i>	[6]	Dismissed
<i>Pseudomonas maltophilia</i>	[7]	Dismissed
<i>Mycobacterium kansasii</i>	[8]	Dismissed
<i>Bacterioides fragilis</i>	[9]	Dismissed
<i>Chlamydia pneumoniae</i>	[10]	Dismissed
Adherent-invasive <i>Escherichia coli</i>	[19-22]	Active
Proteobacteria	[23]	Active
<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>	[24-26]	Active
Viral		
Measles virus	[11,12,14-18]	Dismissed
<i>Cytomegalovirus</i>	[13]	Dismissed

Supporters of the infective theory have considered the possible role of adherent-invasive *Escherichia coli* (AIEC) in IBD etiology. The link between IBD and AIEC was first suggested in 1978 by Tabaqchali *et al.*^[19], who noted high titers of antibodies against AIEC O-antigens in IBD patients. Later, this organism was often isolated from the mucosa of CD patients compared to healthy controls, due to its ability to adhere to gastrointestinal epithelial cells and to demolish the intestinal barrier by producing an α -hemolysin^[20]. Further investigations revealed that AIEC stimulates the release of the pro-inflammatory cytokine, interleukin (IL)-8, which extensively replicates and induces tumor necrosis factor (TNF) α expression in macrophages, leading to the formation of granulomas. In an immunohistochemical study, 57% of tissue samples from CD patients contained *E. coli*, particularly near ulcers, along fissures, around abscesses, within the lamina propria and in the germinal centers of mesenteric lymph nodes^[21]. In another study using polymerase chain reaction (PCR), *E. coli* DNA was found in 80% of intestinal granulomas in CD patients^[22].

A recent review by Mukhopadhyaya *et al.*^[23] extensively described and eventually supported the role of Proteobacteria in the pathogenesis of IBD. They examined the importance of pattern recognition in the extracellular innate immune response of the host and postulated that Proteobacteria with adherent and invasive properties could exploit host defenses, driving proinflammatory changes, altering the intestinal microbiota, favoring dysbiosis and finally leading to the development of IBD.

Several clinical and histopathological similarities between IBD, particularly CD and JD, caused by MAP, indicated a potential role for this pathogen in CD etiology. Similar to *Mycobacterium tuberculosis* or *Mycobacterium leprae*, MAP is able to deregulate immune signaling as one of its survival strategies, resulting in a spectrum of disease manifestations, ranging from simple colonization with a healthy phenotype to severe inflammatory bowel disease^[24]. This association has aroused great scientific interest since the 1980s, when Chiodini *et al.*^[25] isolated MAP and its DNA from biological tissues in CD patients.

Based on these observations, the link between MAP and CD has been widely investigated through direct and indirect methods for MAP detection: PCR for MAP-DNA in blood, stool and biopsies, MAP-specific serological and cell-mediated response, cultures of MAP from intestinal tissues, milk and blood samples^[26].

MAP CHARACTERISTICS AND CLINICAL IMPLICATIONS IN ANIMALS

MAP is a Gram-positive, facultative obligate intracellular bacterial pathogen, acid-fast and dependent on mycobactin for its replication (a liposoluble substance that permits iron utilization). It grows very slowly *in vitro*, taking 8-16 wk or more for duplication, and it appears in human tissue in a cell-wall-deficient state (CWD), also known as “spheroplast”. This is the reason why, different from *Mycobacterium tuberculosis*, it cannot be highlighted by Ziehl-Neelsen staining^[27]. The discrimination between MAP and other organisms belonging to the *Mycobacterium avium* complex (MAC) is known to be problematic. The identification of a species-specific insertion sequence, a fragment of DNA of 1451 base pairs known as IS900, allowed easier distinction using the PCR technique^[28,29]. Through the use of IS900 restriction fragment length polymorphism, two variants of MAP have been identified: type C associated with cattle and S-type, less virulent, associated with sheep. Although these subtypes show a tendency to preferentially infect some animal species, cross-infection is possible and documented^[30].

It is thought that the contagion among animals primarily occurs *via* the fecal-oral route, through the ingestion of milk, colostrum or animal feed contaminated by MAP^[31]. The possibility of intrauterine transmission of this microorganism is also documented, especially in symptomatic animals (20%-40% infected born from a mother with clinically-manifested disease and 10%-20% infected born from a mother with subclinical infection). Once in an organism, MAP is able to invade macrophages in ileal lymphoid tissue and increase in number inside phagosomes, inhibiting their maturation and promoting the recruitment of inflammatory cells. This process, associated with T-lymphocyte activation and interferon (IFN)- γ production, results in granulomatous lesions that characterize JD, or paratuberculosis, a chronic widespread enteritis of cattle, sheep and other ruminants. The progression of disease is classically categorized into four stages: silent infection, subclinical stage, clinical stage and clinically evolved disease. Animals are generally infected at an early age. Clinical signs, dominated by diarrhea and weight loss, generally occur 3-5 years after infection. This long-lasting incubation period depends on the level of environmental contamination (MAP exposure dose), virulence of the MAP strain, the capacity of a single animal of hinder the infection (immune response is more efficient with age), and genetic susceptibility *vs* MAP (inter- and intra-species differences)^[32]. MAP has been isolated from the feces and milk of ruminants with clinical

and subclinical infection: this fact explains the wide diffusion of MAP infection and the vast environmental contamination^[33]. MAP can survive for a long period in different ecosystems, due to its high environmental resistance, but replication outside infected animals is not possible due to its dependency on mycobactin. Several studies have been carried out to identify environmental sources of human exposure to MAP, including its presence in water supplies (domestic water and rivers)^[34-36], milk, dairy products, and even in meat^[37]. MAP's ability to spread *via* the milk of infected animals has been proven and it has been shown that this microorganism is able to survive routine pasteurization methods, with persistence of vital mycobacteria and antigenic components in commercial milk^[38]. MAP is widely present in the human food chain, and even in dairy products representing a potential exposure source. Environmental diffusion of MAP could represent a favorable condition for IBD onset when associated with intestinal immunodeficiency in susceptible subjects^[39].

“MAP HYPOTHESIS”: SUPPORTING AND CONTRASTING EVIDENCE

The MAP hypothesis in CD is considered to have originated in the 1980s, when Chiodini *et al.*^[25] demonstrated that MAP isolated from human tissues can cause a granulomatous disease of the distal small intestine in experimental animals. The authors isolated from the intestinal tissues of two CD patients a mycobacterium which shared the acid-fast and mycobactin-dependent characteristics of MAP. When injected intravenously or intraperitoneally in experimental animals, this organism induced a disease characterized by hepatic and splenic granulomas in mice, but not in rats, guinea pigs, rabbits or chickens. It also resulted in granulomatous disease in the distal small intestine in young goats, when administered orally. Acid-fast bacilli were not found in autopsy sections of the intestine, however, a single organism with characteristics similar to the one administered was seen in microgranulomas of the mesenteric lymph nodes. The identification of MAP in human tissues is seldom reported, because of its CWD form, as Chiodini *et al.*^[25] stated. Two recent meta-analyses showed that the association between MAP and CD seems to be specific compared with individuals free of IBD. The pooled Odds Ratio from studies using PCR for tissue samples was 7.01 (95%CI: 3.95-12.4) and was 1.72 (95%CI: 1.02-2.90) in studies using enzyme-linked immunosorbent assay (ELISA) for serum^[40,41].

Indirect support for the “MAP hypothesis” comes from Shanahan *et al.*^[42] who found a similarity with *Helicobacter pylori*, an infectious agent widely dispersed, but able to cause peptic disease and gastric cancer only in a minority of exposed subjects. Similarly, *Mycobacterium tuberculosis* affects about one third of the world's population, but only 5%-10% develop clinically manifested disease. Analogies exist for MAP, in that it is widely dispersed and affects animals and humans, possibly playing a role in CD

Table 2 Direct methods for detection of *Mycobacterium avium* subspecies *paratuberculosis*

Method	Tissue analyzed	Ref.	+/-	Advantage	Defect
MAP culture				Effective isolation of microorganism	Organism's fastidious nature; slow-growth
	Intestinal biopsy	[55]	+		
	Peripheral blood	[57,59,60]	-		
PCR for MAP-specific IS900 DNA				Fast and easy isolation of MAP fragment	Not able to distinguish vital MAP <i>vs</i> fragment of killed MAP; confounded by frequent MAP opportunistic infection (oro-fecal route)
	Intestinal biopsy	[53-55]	+		
	Peripheral blood	[58]	+		
	Peripheral blood	[59,60]	-		
	Stools	[63]	-		
PCR for MAP-specific IS900 DNA				Demonstrate the presence of active/vital metabolic processes	Difficult to reproduce (RNA half-life measured in min)
	Intestinal biopsy	[62]	+		

+: Supporting *Mycobacterium avium* subspecies *paratuberculosis* (MAP) hypothesis; -: Contrasting MAP hypothesis; PCR: Polymerase chain reaction; IS900: Insertion sequence 900; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid.

onset only in few subjects.

A recent study by Xia *et al.*^[43] showed significantly higher levels of antibodies against a specific mycobacterial protein, tyrosine phosphatase (PtpA), in CD patients when compared to healthy controls. PtpA is secreted during MAP infection in a time-dependent manner from the human-derived monocyte cell line THP-1. The same evidence was not observed in UC patients, suggesting the exclusive involvement of MAP in CD^[43].

Although analogies exist between CD and JD, some discrepancies should be mentioned. JD does not present some of the basic patterns of CD: the segmental localization and features that can complicate the evolution of CD, such as fistula, fissures, bleeding, stenosis, adhesions, perforation and the formation of abscesses are not described in JD.

Against the "MAP hypothesis", immunosuppressive drugs, widely employed in IBD, should not be used in latent or clinically manifested mycobacterial infections, as they increase the risk of onset and/or worsening of the disease. Screening analyses are necessary to exclude any type of mycobacterial infections before administering anti-TNF α drugs. *Mycobacterium tuberculosis* massively proliferates with anti-TNF α drugs or steroid treatment, and the same for *Mycobacterium avium intracellulare* in the intestine of HIV-infected patients^[5]. It is possible that intracellular cell wall deficient MAP may not be able to replicate despite ongoing immunosuppressive therapy. Corticosteroid therapy was associated with lower MAP detection rates in the study by Autschbach *et al.*^[44].

In patients treated with infliximab, a decreased level of antibodies against PtpA was found and no statistical differences were observed in comparison to the controls. This observation suggests that inhibition of TNF α may reduce MAP growth probably with a negative effect on the secretion of PtpA^[43,45,46]. Alternatively, infliximab interferes with the antigen presentation system in macrophages dampening the immune B response and the antibody titer. In this respect, a statistical difference in antibody titer was observed between CD patients treated with azathioprine (AZA) and untreated patients; no significant difference was observed when patients were treated with either 5-ASA or steroids^[43]. "In vitro" studies

showed the ability of AZA^[47] and other immunosuppressants, such as cyclosporine^[48] and methotrexate^[49], to inhibit MAP growth in the culture system.

In addition, epidemiological evidence supporting the association between MAP and CD is still lacking. The prevalence of IBD is more common in urban areas, while no increased incidence of disease was detected among people in rural areas, especially in farmers and their families, despite major exposure to MAP^[50]. Furthermore, environmental conditions which should promote MAP transmission, such as poor hygienic conditions and overcrowding, seem to protect against CD^[51,52]. The lack of evidence for horizontal or vertical transmission of CD leads to MAP being a zoonotic agent or an opportunistic pathogen in humans.

It is hard to explain the paradox that an infection, although able to stimulate such an intense inflammatory reaction within the gut in CD patients, is not associated with a strong cellular or serologic reactivity against MAP. Detection of MAP-DNA in the granulomas of intestinal CD is neither disease-specific nor bacterial-specific, because other forms of bacterial DNA are also present^[22]. IS900 was scientifically proven to be species-specific in animals affected by JD, however, its specificity in human tissues or in environmental ecosystems is unknown. Past studies supporting the MAP hypothesis probably included a percentage of false positive findings, ascribable to other mycobacterial species. IS900 PCR should be used as a confirmation test, in addition to other methods able to detect MAP presence in tissues.

DIRECT METHODS FOR MAP DETECTION

Because of its presence in human tissues in an intracellular cell-wall-deficient shape, MAP cannot usually be identified by Ziehl-Neelsen staining. The direct detection of MAP in humans requires culture, PCR for MAP-DNA/RNA (Table 2).

Isolation of the microorganism by culture methods is considered the gold standard for MAP detection. This method remains problematic and time-consuming even in the best circumstances, due to the organism's fastidious nature and slow growth. Although the use of MGIT

(Mycobacterial Growth Indicator Tube - Becton Dickinson) reduces growth time to 10-12 wk, there are still difficulties in culture isolation. DNA amplification with PCR and *in-situ* hybridization are the two main methods for MAP detection, using the IS900 sequence as a target.

Several studies, based on cultural methods and/or molecular techniques, demonstrated a higher prevalence of both the microorganism and its DNA in CD patients compared to controls. The presence of MAP has been investigated in different tissues, such as intestinal biopsies and surgical specimens, blood and recently fecal samples.

Biopsy specimens have always been considered by researchers as irreplaceable biological samples and have been widely utilized for MAP detection. Sanderson *et al.*^[53] identified MAP IS900 DNA in 26 of 40 (65%) CD patients, 1 of 23 (4.3%) UC patients and 5 of 40 (12.5%) control subjects. Later, Bull *et al.*^[54] described a MAP-DNA prevalence of 92% in CD patients (34 of 37) *vs* 26% non-IBD controls (9 of 34) ($P = 0.0002$). In 2005, Sechi *et al.*^[55] found a highly significant difference between CD patients (83.3%) and the control group (10.3%) ($P = 0.000001$) for IS900 PCR presence in DNA extracts of fresh intestinal mucosal biopsies, as well as in culture using supplemented MGIT media (63.3% and 10.3%, respectively, $P = 0.00001$). In the same year, Autschbach *et al.*^[44] and Romero *et al.*^[56] published data on the prevalence of MAP in surgical tissues.

Several authors have investigated the presence of viable MAP through culture or the detection of MAP-DNA in peripheral blood using PCR. Naser *et al.*^[57] found a statistically significant positivity in the CD group compared to the control. This result was confirmed in the study by Bentley *et al.*^[58], who found a significant overrepresentation of MAP-DNA in patients with CD *vs* controls (33.8% *vs* 21.5%, $P = 0.002$) in a larger cohort. Two recent meta-analyses have been published showing a generally higher MAP prevalence in CD *vs* controls in different samples^[40,41].

However, a number of investigations have failed to demonstrate any evidence of an association between CD and detection of MAP-DNA. Parrish *et al.*^[59] reported that all CD patients were negative for MAP using PCR and only one healthy control patient was positive; however, no viable MAP was cultured from this individual and all blood cultures were negative for MAP, despite the large study size (130 CD, 130 healthy controls). Other authors found a high prevalence of MAP even in non-IBD controls^[60].

Molecular techniques for assaying MAP-DNA are not able to discern the presence of vital microorganisms from fragments of killed MAP. RT-PCR for IS900-RNA detection overcomes this problem by directly demonstrating the presence of active metabolic processes at the time of isolation^[61]. The most significant study in this field, although limited to a small number of patients (CD $n = 8$, UC $n = 2$, and controls affected by colon-rectal cancer $n = 2$), identified the presence of MAP-RNA in all ileal mucosal samples from CD and UC patients^[62]. RNA has a

half-life of minutes, therefore, detection of this molecule cannot only be related to the wide environmental spread of MAP, as would be legitimate to suppose in the case of DNA detection.

Recent studies have investigated the presence of IS900 DNA fragment in stools from CD patients, UC patients and from healthy controls. The use of human feces represents a new approach to investigate the "MAP hypothesis"; as it has the advantage that stools can be collected more easily than blood samples and biopsy specimens. In a previous study, the results revealed that detection of MAP DNA in human feces is very common: 21 of 31 CD (68%), 13 of 20 UC (65%) and 11 of 23 healthy controls were MAP-positive [CD *vs* controls: $P =$ no significance (NS), UC *vs* controls: $P =$ NS]^[63]. The IS900-positive percentage in IBD samples was higher than in healthy controls, but did not reach statistical significance, partially due to the small sample size. No significant correlation was observed when analyzing IS900 positivity in relation to disease activity in the IBD group^[63].

INDIRECT METHODS FOR MAP DETECTION

Besides direct methods for MAP identification, indirect techniques are also available, with the aim of testing humoral and cellular-mediated immune response using the blood of IBD patients and healthy controls (Table 3).

ELISA represents the main diagnostic method for identifying cattle affected with JD, and is the most frequently used method to identify subjects immune to MAP. However, an ELISA validated for humans is not presently available.

Many studies have assayed the presence of MAP antibodies in CD patients and controls, obtaining contrasting results that should be related to the use of different methodologies and heterogeneous antigens (nuclear extracts, soluble antigens from cultured filtrates, recombinant antigens). For example, 57% of CD patients were positive for the detection of antibodies against a 38-kDa antigen, while 53% manifested specific antibodies for 24-kDa and 18-kDa bacterioferritin, but only 18% showed simultaneous seropositivity for all the three antigens^[64]. Other studies demonstrated that the detection of anti-p36 antibodies is usually positive in CD patients (about 89%), while it seems to be less common in UC patients (about 15%). A specific reactivity against p35 and p36 is frequent in CD patients, but both these antigens are common to *Mycobacterium avium* subspecies *avium*, suggesting that cross-reactivity may influence results^[65]. Other mycobacterial components used for testing humoral response against MAP are 14-kDa AhpC antigen and the heat shock proteins^[66]. Of 13 studies included in a recent meta-analysis, 10 found that the prevalence of antibodies against MAP was higher in patients with CD than in controls, with a pooled odd ratio of 1.72 (1.02-2.90); similar pooled odd ratios were obtained when CD and UC pa-

Table 3 Indirect methods for detection of *Mycobacterium avium* subspecies *paratuberculosis*: humoral and cellular mediator of immune response to *Mycobacterium avium* subspecies *paratuberculosis*

Method	Tissue analyzed	Antigen/cellular mediator	Ref.
ELISA for antibody detection	Peripheral blood	38-kDa, 24-kDa, 18-kDa	[64]
		p35 <i>vs</i> p36	[65]
		14-kDa, AhpC, HSP	[66]
		MAP protoplasmic antigen	[67]
		18-kDa	[68]
		Anti-PPA	[69]
		Ptp-A	[43]
		Ptp-A, protein kinase G	[46]
ELISA for cytokine detection (proliferative assay)	Peripheral blood lymphocytes	INF γ ; IL-17	[71,72]
	Gut biopsies lymphocytes T CD4 ⁺	INF γ ; IL-17	[73]
	Peripheral blood monocyte THP-1	TNF α	[74]

ELISA: Enzyme-linked immunosorbent assay; AhpC: Alkyl hydroperoxide reductase C; HSP: Heat shock proteins; PPA: Ptp-A protein tyrosine phosphatase; INF- γ : Interferon-gamma; IL-7: Interleukin 7; TNF α : Tumor necrosis factor alpha.

tients were compared (1.88; 1.26-2.81)^[41].

Nevertheless, other studies on humoral response against MAP antigens did not find an association between CD and MAP: Brunello *et al*^[67] reported no significant differences in the titers of IgG antibodies against MAP protoplasmic antigen between CD (3.7%) and UC patients (5%). Walmsley *et al*^[68] determined anti-18 kDa mycobacterial bacterioferritin IgG or IgA titers in CD patients and controls without finding any differences, while the results from Bernstein *et al*^[69] showed a seropositivity rate for anti-PPA (anti-purified protoplasmic antigen) in 37.8% of CD patients, 34.7% of UC patients and 33.6% of healthy controls, with non-significant statistical differences among the groups.

Patients with IBD have an impaired barrier function of intestinal mucosa with increased bacterial translocation^[70]. In relation to these data, increased antibody titer against *E. coli*, aerobes, anaerobes and enteric bacterial pathogens has been reported both at the mucosal and systemic level^[21]. Thus, a pronounced humoral and cell-mediated immune response against bacteria is unavoidable. The same could be true for MAP, however, this is not enough to support its etiopathological role in CD onset.

Moreover, the cell-mediated response against MAP, dosing INF- γ and other inflammatory cytokines, have also been analyzed^[71,72].

In-vitro INF- γ lymphocyte production, derived from peripheral blood after stimulation with MAP PPD-antigen, was simultaneously evaluated with other MAP detection methods (PCR for MAP-DNA in tissues, direct culture isolation, ELISA for MAP-specific antibodies and cell-mediated response). The authors observed higher levels of MAP-specific INF- γ in the control group compared to IBD patients, supporting the hypothesis of a wide environmental diffusion of MAP and that healthy subjects could have a stronger cell-mediated immune response, better than the IBD patients.

However, Olsen *et al*^[73] found that isolated T CD4⁺ clones from gut biopsies of patients with active CD

significantly proliferate in response to MAP-specific antigens *vs* controls and were able to produce INF- γ and IL-17.

Protein tyrosine phosphatase (PtpA) and Protein Kinase (PknG) are a very recent indirect method of detecting the presence of MAP. Both proteins are involved in the signal transduction system of MAP and seem to have a critical role for survival of the pathogen within human macrophages, modulating the host's immune response^[45]. PtpA, is a specific mycobacterial protein tyrosine phosphatase secreted in a time-dependent manner during MAP infection from the human-derived monocyte cell line, THP-1, and can inhibit phagosome acidification and phagosome-lysosome fusion by dephosphorylating the host sorting protein VPS33B. When sera from CD and UC patients and controls were screened for these antibodies, there was a significantly higher titer in CD patients *vs* controls. In contrast, no significant difference was measured in UC patients^[43].

Nakase *et al*^[74] demonstrated that THP-1 cells infected with MAP produced a higher amount of TNF α when compared with either *Mycobacterium avium* or *Mycobacterium smegmatis*, suggesting that MAP is directly involved in the up-regulation of this cytokine.

ROLE OF COMBINED

ANTIMYCOBACTERIAL THERAPY IN CD

In animals, JD is a chronic progressive fatal condition. Not even pre-emptive administration of antibiotics prevents the infection. *In vitro* analysis showed that MAP is not responsive to traditional anti-tuberculous drugs. In addition, *in vivo* the association of isoniazid, ethambutol and rifampicin lacks efficacy in patients with CD, as demonstrated by a two-year clinical trial, followed by a three-year follow-up period^[75]. Because of its typical CWD form, antibiotics that interfere with bacterial wall synthesis do not show efficacy against MAP, which is similar to drugs that impede bacterial duplication at different levels, as MAP

has a low replication rate. Combined antibiotic therapies, including rifampicin and macrolide-derived drugs, for instance clarithromycin and azithromycin, demonstrated *in-vivo* and *in-vitro* efficacy both against MAP and other members of the *Mycobacterium avium* complex^[76]. Similar to *Mycobacterium tuberculosis*, the importance of a therapeutic scheme consisting of at least three drugs in order to prevent drug-resistance was also confirmed for other mycobacterial species.

It has been suggested that the most irrefutable evidence of the involvement of a microbial agent in CD etiology would be the long-term remission of clinical manifestations following the clearance of infection^[5]. With regard to this issue, Borody *et al*^[77] treated a series of 12 patients suffering from severe, obstructive or penetrating CD with a triple antibiotic therapy consisting of rifabutin, clofazimine and clarithromycin, and obtained a clinical, endoscopic and histological remission rate of almost 50%. In an English clinical trial based on the use of rifabutin, clarithromycin and azithromycin from 6 to 35 mo, with a follow-up of 7 to 41 mo, therapy was tolerated by 46 of 52 (89%) enrolled patients; in particular, 17 of 19 patients (91%) who were steroid-dependent before treatment were steroid-independent. Furthermore, the authors observed an important improvement in CDAI, maintained over the following 24 mo^[76]. In an American clinical trial, 36 patients positive for p35 and p36 MAP-specific antigens were followed for a period of 4-17 mo; 21 of 29 CD patients (58.3%) who seemed to tolerate antibiotic therapy with rifabutin and clarithromycin showed a clinical sustained improvement^[78].

These results seem promising, suggesting that at least a sub-group of CD patients benefit from antibiotic treatment. Nevertheless, these trials are not controlled, are based on a small number of patients and with variability concerning the antibiotics used. Selby *et al*^[79] published the result of a large controlled trial in which 213 patients with active CD were randomized to receive triple intracellularly active antibiotic treatment (clarithromycin, rifabutin, clofazimine) or placebo for two years, followed by one year of follow-up, in addition to a 16-wk tapering course of steroids. They observed a relapse rate of 59% in the antibiotics arm *vs* 50% in the placebo arm ($P = 0.54$) among the 32 patients who completed the study at the end of the two-year period. No statistically significant differences between the two arms of the study were noted either during the two-year treatment, or during the follow-up period.

Incongruity of these results may be due to the heterogeneity of the patient selection criteria, the drug type and dosage, the use of mono-therapy or combined therapy and the duration of treatment. Primary end-points were different (drug-capacity of inducing and/or maintaining clinic and/or endoscopic disease remission, relapse percentages after treatment suspension) with consequently different interpretation of the results. Furthermore, none of the previous studies included evaluation of MAP presence in the enrolled population. The eradication of

MAP after antibiotic therapy would strongly support the role of MAP in CD etiology.

FINAL CONSIDERATIONS AND PERSPECTIVES

The present data do not demonstrate that MAP is the causal agent in CD, however, a certain degree of involvement of this bacterium in the physiopathological steps of the disease is reasonable. The literature contains contrasting and contradictory evidence on this association with a lack of uniformity in the materials and methods adopted by different researchers for MAP detection and inappropriate patient and control selection parameters. The variability in quality and quantity of analyzed samples, the possible contamination during collection, transport and/or manufacturing of samples, produced further bias among studies^[80]. In order to consider an infectious agent as the cause of disease, it is necessary to demonstrate that infection precedes the disease onset. Even studies on the causative role of MAP in CD did not sufficiently support this association because they did not provide enough evidence between the timing of infection and the onset of intestinal disease. The detection of MAP-DNA in the blood of CD patients might suggest that a viable form of the organism is present in CD; however, this could be a secondary phenomenon due to increased intestinal permeability and/or the inability of macrophages to kill MAP in CD patients, rather than an etiological explanation for the disease.

To further elucidate the role of MAP in CD and IBD it would be useful to simultaneously evaluate different biological samples (blood, biopsies and stools) and to clarify whether the detection of MAP-DNA in stools or in biopsies may be related to its viable presence in blood, representing a possible marker of active infection. To our knowledge only two small studies on this issue have been published to date^[71,72].

There is no effective and readily accessible method available to diagnose MAP-infection in humans. CD histology suggests that the host's immune response is primarily cell-mediated. Therefore, studies which investigate cell-mediated reactivity against MAP, through the use of T-cell-based IFN- γ assays, could gain more relevance. The reliability of the quantiferon-TB Gold test in evaluating the contact with non-tubercular mycobacteria is still in doubt. The positive cut-off level for the Quantiferon test in patients with non-tuberculous mycobacterial disease has not been determined^[81]. The development of "home-made" INF- γ release assays that specifically dose the quantity of this cytokine after stimulation with MAP-specific antigen, including proper negative and positive controls, is required.

As demonstrated, steroid therapies and combinations with immunosuppressive agents increase the risk of an indeterminate result in the Quantiferon test for the detection of latent tuberculosis infection^[82]. We know that

MAP infected human macrophages disrupt the host's immune response for its own benefit and a high amount of the cytokine, TNF-alpha, was found in MAP associated CD patients^[74].

The controversy regarding MAP and IBD has persisted far too long. Firstly, it is necessary to ratify criteria for sample collection, test performance and interpretation of results. Secondly, in order to establish a causal role of MAP in the etiology of CD, it is necessary to determine if clearance of MAP using drugs that specifically act against this organism, selectively change the natural history of the disease, guarantee a sustained clinical remission and an improvement in histological activity.

The interest in a possible link between MAP and CD would be of clinical relevance (development of diagnostic methods) and for the prevention of the disease (implementation of public health measures, modifications in food processing practices, develop screening MAP infection).

Heterogeneous clinical and histological features, disease course and response to therapies make CD a highly polymorphic entity^[83] and is better identified as a "syndrome". In this respect, CD may not have a singular etiology, but rather result from the concomitant action of multiple causal agents and triggering factors, including MAP.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Surgical treatment of ulcerative colitis: Ileorectal vs ileal pouch-anal anastomosis

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Core tip: Ileal pouch-anal anastomosis (IPAA) is the most commonly performed procedure for treatment of UC patients refractory to medical therapy. However, IPAA carries on its own risks. Recently, some authors have proposed ileorectal anastomosis (IRA) as a valid surgical alternative to IPAA. IRA is an easier operation than IPAA associated with low complication rates and comparable long-term functional results. This manuscript reviews the pros and cons of both procedures and compares results.

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Abstract

Total proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the current gold standard in the surgical treatment of ulcerative colitis (UC) refractory to medical management. A procedure of significant magnitude carries its own risks including anastomotic failure, pelvic sepsis and a low rate of neoplastic degeneration over-time. Recent studies have shown that total colectomy with ileorectal anastomosis (IRA) has been associated with good long-term functional results in a selected group of UC patients amenable to undergo a strict surveillance for the relatively high risk of cancer in the rectum. This manuscript will review and compare the most recent literature on IRA and IPAA as it pertains to postoperative morbidity and mortality, failure rates, functional outcomes and cancer risk.

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INTRODUCTION

The main goals of surgical treatment for ulcerative colitis (UC) are not only to alleviate symptoms and minimize cancer risk but also to obtain good functional outcomes and improve quality of life. Until the 1950s total proctocolectomy with end-ileostomy (TPC) was the only available procedure for UC patients failing medical management.

In the 1940s reports of subtotal colectomy with ileorectal anastomosis (IRA) as an alternative to TPC in selected patients were first published^[1]. During the 1950s and 1960s, Aylett^[2] became the leading proponent of this procedure describing it as a way to avoid a permanent stoma. At that time IRA represented a valid alternative to TPC in high selected patients with minimal rectal inflammation. It was a less invasive operation, performed in one

Table 1 Morbidity and mortality after ileorectal anastomosis

Series	Period	n	Anastomotic leak (%)	Proctitis (%)	Need for proctectomy (%)	Overall morbidity (%)	Mortality (%)
da Luz Moreira <i>et al</i> ^[11]	1971-2006	86	2.3	28.0	53	8	0
Leijonmarck <i>et al</i> ^[13]	1955-1984	51	3.9	45.1	57	16	4
Pastore <i>et al</i> ^[15]	1974-1990	48	4.4	10.4	17	22.9	0
Börjesson <i>et al</i> ^[16]	1997-2003	32	3.1	9.3	12	28	0
Grundfest <i>et al</i> ^[17]	1957-1977	89	9.1	11.2	21	16.1	0
Elton <i>et al</i> ^[18]	1990-1999	18	5.6	11.0	17	22.2	0
Andersson <i>et al</i> ^[19]	1992-2006	105	2.8	8.6	13.3	12.4	0
Lepistö <i>et al</i> ^[22]	1978-2000	20	-	45.0	35	-	0
Oakley <i>et al</i> ^[23]	1960-1982	288	-	41.0	55.2	-	4.2

stage and not requiring pelvic dissection with the associated risk of sexual dysfunction^[3-5].

In 1978, Parks *et al*^[6] described an ileal pouch-anal anastomosis (IPAA). Since then, IPAA has become the procedure of choice for patients affected by UC with excellent long-term functional results, low risk of persistent cuff inflammation or neoplastic degeneration in the retained rectum^[7,8]. Consequently, many surgeons have abandoned IRA in favor of IPAA and TPC has remained an option for patients not candidates for IPAA. The counterargument is that IPAA, as a major procedure, carries its own risks including anastomotic failure and pelvic sepsis that could result in poor pouch function, pouchitis and infertility in young women, as well as pelvic nerve damage and portal vein thrombosis^[3-5,9,10]. In addition few cases of cancer have been reported arising not only in the anal transitional zone but also in the pouch itself^[7,8].

Interestingly, recent series^[11-14] of selected UC patients undergoing IRA showed long-term functional results similar to IPAA.

The aim of the current study was to review and compare the most recent literature on IRA and IPAA as it pertains to postoperative morbidity and mortality, failure rates, functional outcomes and cancer risk. It will help surgeons to provide a tailored treatment for UC patients.

ILEORECTAL ANASTOMOSIS

Chronic UC begins in the rectum and extends proximally in a continuous fashion. The severity of the disease also seems to be higher distally with the exception of fulminant pancolitis presentation. However, distal disease is sometimes alleviated by topical treatment and patients with minimal rectal involvement and no dysplastic changes in the rectum could be considered for IRA. Furthermore, an adequate rectal compliance and a normal anal sphincters function are critical for good long-term results. These functions can easily be assessed by digital rectal examination but more accurately by rigid/flexible proctoscopy and manometry. Patients with poor sphincter function, severe rectal disease, and non-distensible rectum should not be offered an IRA. On the contrary, patients with colitis associated colorectal cancer and advanced metastatic disease may benefit from an IRA because of their short life expectancy and the palliative

nature of their treatment.

Several studies^[11,13,15-17] have shown IRA for UC to be safe, with low postoperative morbidity and mortality. During the years, overall morbidity has been reported between 8% and 28% and mortality between 0% and 4% (Table 1). These studies including the work of Elton *et al*^[18] and Andersson *et al*^[19], focused their attention on postoperative complications including small bowel obstruction, anastomotic leak and abdominal abscess. The fatal events were due to anastomotic leak and subsequent sepsis and to a pulmonary embolism.

The majority of published data has included mainly primary anastomosis with leak rates ranging from 2% to 9%^[11,13,15-20]. Diverting ileostomies have been utilized in selective cases at the surgeon discretion. Turnbull^[21] suggested that preservation of grossly involved rectosigmoid colon was the main cause of IRA failure. In his opinion an anastomosis at 6 cm or less above peritoneal reflexion improved rectal inflammation during the first months and reduced the likelihood of IRA failure.

IRA does not involve extensive pelvic dissection, unlike IPAA or TPC, minimizing the risk of sexual and urinary dysfunction. Hence, higher fertility rates may be expected in IRA patients compared to IPAA although definitive studies providing evidence for better fertility rates in UC patients are lacking. Thus, colectomy with IRA could be considered when treating women in their reproductive age^[20].

Some authors^[2,11,13,15,18,22] have shown acceptable long-term success rate after IRA. Aylett^[2] in 1966 reported on a total of 300 cases operated on over a ten-year period with only 7% failure rates. Lepistö *et al*^[22] and Pastore *et al*^[15] reported a cumulative probability of having a functioning IRA at five years of 84%. Elton *et al*^[18] had 88% success in their 18 patients, but the follow-up in that study was shorter. Ten year cumulative success (69%) in Lepistö's series^[22] was higher than reported by Leijonmarck *et al*^[13] (51%) in 1990. At 20-years the current probability of having a functioning IRA has ranged between 46% and 69%^[20]. One recent study proposed by da Luz Moreira *et al*^[11], from Cleveland Clinic, compared 22 IRA with 66 IPAA patients matched for age, gender, and follow-up time, including IRAs performed in the past 25 years showed a cumulative probability of having a functioning IRA at 5, 10, 15 and 20 years of 81, 74, 56

and 46 percent respectively in accordance with previously published work.

In terms of functional results, the da Luz Moreira's series^[11] reported six bowel movements per day (range 2-11), 1/22 (5%) night-time seepage and 15/22 (68%) reporting frequent urgency. Leijonmarck *et al*^[13] showed four bowel movements per day and none during the night, with 100% of continence (25% of patients are on antidiarrheal medication), after a mean follow-up of 13 years. Elton *et al*^[18] had no significant difference between preoperative and 1-year postoperative stool frequency, 11/12 patients had no problems with continence, and three were using antidiarrheal medication. Pastore *et al*^[15] described a median number of six bowel movements per day (range 2-20). The median number of nocturnal bowel movements was one (range 0-10) and three patients had more than eight daily stools with frequent soiling and urgency. At the time of follow-up, antidiarrheal medications were taken by 53.3% of patients, whereas 31.3% required low doses of systemic or topical steroids. More than 90% of patients considered that their health status had improved after the operation. Quality of life was improved in 84%.

All the studies above showed that IRA is a safe procedure with an acceptable function and quality of life but unfortunately it is not necessarily a definitive operation, especially for young patients. Specifically, Andersson and colleagues^[19] reported an estimated cumulative failure rate of 10.1% and 24.1% respectively at 5 and 10 years. In his series Leijonmarck *et al*^[13] had 57% of failure after 13-year follow-up. Pastore *et al*^[15] suggested that time between IRA and additional surgery in his series was 3.9 ± 4.7 years. In da Luz Moreira's series^[11], 38 patients (44%) continued to have a functioned IRA after a median follow-up of 11 years (range 1-30 years). The rectum was resected in 46 (53%) of 86 patients and the median follow-up between IRA and completion proctectomy was 10 years (range 1-33 years).

The main indication for proctectomy is recurrent proctitis refractory to medical management^[11,13,15-19,22,23], followed by dysplasia or cancer, and the development of Crohn's disease. The options for these patients include IPAA, Brooke ileostomy, or a continent ileostomy (Kock pouch). Very Often IPAA can be safely performed in the majority of these patients thus preserving bowel continuity and avoiding permanent fecal diversion^[11].

Cancer risk

Mucosal dysplasia is a premalignant pathological state associated with long standing UC^[24]. Dysplasia in general is considered an indication for surgery in UC, even though this paradigm is rapidly changing. Epithelial dysplasia of the colon and rectum was graded as mild, moderate, or severe depending on whether the upper one-third, upper two-thirds, or entire glands displayed nuclear anisocytosis and hyperchromatism, as well as loss of nuclear polarity and the normal goblet cell configuration of colonic mucosa. Since dysplastic changes were often patchy, only

the highest degree of dysplasia was considered. Johnson *et al*^[25] had shown in 1983 that the probability of developing rectal adenocarcinoma after a diagnosis of mild or severe dysplasia in IRA patients reached 42% at nine years from diagnosis. The rate of dysplasia and cancer, in patients with UC, increases with time and leaving the rectum in place contributes to the increased risk. The overall cumulative probability of rectal dysplasia in the retained rectum increases from 9% at 10 years to 25% at 20 years^[11]. The overall incidence of rectal cancer after an IRA varies in the literature based on length of follow-up, ranging from 0% to 18%. Grundfest *et al*^[17] reported on four patients who developed carcinoma of the rectum during their study period (4.8% at 8-year follow-up), although he estimated the risk of rectal cancer to be 13% at more than 25 years of follow-up. Oakley *et al*^[23] found nine patients with rectal cancer in the stump (3.1%) at an 8-year follow-up while Andersson *et al*^[19] showed an overall risk of cancer of 1.9% at a 5.4-year follow-up. However, some series reported higher rates of degeneration as Baker *et al*^[26] who described, in 1978, a cumulative cancer risk of 6% after 20 years rising to 18% after 35 years in a series of 374 unselected patients. In da Luz Moreira's series^[11], the cumulative probability of developing dysplasia and cancer was 7%, 9%, 20% and 25% and 0%, 2%, 5% and 14% at 5, 10, 15 and 20 years respectively. On the other hand, Leijonmarck *et al*^[13] and Lepistö *et al*^[22] had reported no case of cancer in more recent series at 13 and 18-year follow-up, respectively. Pastore *et al*^[15] showed a cumulative probability of remaining free of cancer around 85.5% at 12 years (95%CI: 57.7%-100%).

Most patients who develop rectal cancer in the retained rectum presented at an advanced stage (stage III-IV) suggesting the possibility of a more aggressive biology and making close surveillance imperative^[11,27]. For instance, in Baker's study 62% of patients who had developed rectal cancer died within three years of diagnosis. Johnson *et al*^[27] reported a total of 10 rectal cancers, 8 of which had either nodal or distant metastases. The patients in the series reported by Oakley *et al*^[23] fared better, with just 2 of 9 patients with rectal cancer dying over a 22-year time period. Rectal biopsies taken from multiple sites every 6 to 12 mo are advised following IRA in UC patients. If dysplasia is found, completion proctectomy is indicated. Patients with long standing UC who are not able or willing to undergo surveillance should not be offered an IRA. It is also important to emphasize that colectomy with IRA should not be offered to patients with preexisting dysplasia or cancer due to the increased risk of further neoplastic degeneration^[28]. In addition, the presence of dysplasia or cancer in the resected colon should cause particular concern about the fate of the remaining rectum suggesting that a completion proctectomy would be indicated in these cases. In fact, Oakley *et al*^[12] reported on five surviving patients who had cancer in their colonic specimens; three of the five were found on follow-up to have cancer or severe dysplasia in the rectal remnant. Grundfest *et al*^[17] described nine patients

Table 2 Morbidity and mortality after ileal pouch-anal anastomosis

Period	No. of studies	No. of patients	Pelvic sepsis (%, 95%CI)	Pouch failure (%, 95%CI)	Pouchitis (%, 95%CI)	Mortality (%, range)
Meta-analysis studies < 2000 ^[32]	43	9317	9.5 (8.2-10.9)	6.8 (5.8-8.4)	18.8 (15.7-22.4)	-
Meta-analysis studies ≥ 2000 ^[33]	53	14966	7.5 (6.1-9.1)	4.3 (3.5-5.3)	26.8 (21.0-33.5)	0 (0-2.9)

with a colitis-associated colon cancer or severe dysplasia who underwent subtotal colectomy, eight of whom survived; of the eight, five developed severe dysplasia or cancer in the retained rectum.

ILEAL POUCH-ANAL ANASTOMOSIS

Restorative proctocolectomy with IPAA is currently the procedure of choice for the surgical treatment of UC. The main reason for its popularity is its avoidance of a permanent stoma with stable functional results and good quality of life. In over 30 years of its existence, the IPAA has undergone several refinements in the quest of achieving optimal results. Examples include different shapes of the pouch, different anastomotic techniques, use of defunctioning ileostomy and various dissection methods^[29-31]. Surgeons have also obtained greater experience and familiarity with the technique, which has also benefited outcomes.

A large body of literature exists on the outcomes of IPAA. Most studies, however, are retrospective cohorts reporting outcomes from a single institution. Due to large variations between studies, an overview is needed for reliable assessment of the IPAA outcomes. A meta-analysis of 43 observational studies, all published before 2000, has provided pooled estimates of complications and functional outcomes after IPAA^[32]. This meta-analysis showed a pouch failure risk of 6.8% (95%CI: 5.4%-8.4%), increasing to 8.5% (95%CI: 5.4%-13.2%) when only patients with a minimal follow-up of 5 years were considered^[32]. Other pouch related complications were also studied. Pelvic sepsis and pouch fistulas, both major post-operative complications, were observed in 9.5% (95%CI: 8.2%-10.9%) and 5.5% (95%CI: 4.3%-7.0%), respectively. Sexual dysfunction was present in 3.4% (95%CI: 2.7%-4.7%), while pouchitis was reported in 18.8% (95%CI: 15.7%-22.4%).

A recent meta-analysis, including 53 studies published after 2000, showed significant improvements in these results^[33]. The overall rate of pouch failure was significantly reduced to 4.3% (95%CI: 3.5%-5.3%), and pouch failure after at least 5 years of follow-up was 4.7% (95%CI: 3.4%-6.4%). An improvement was also seen in most other complications. Pelvic sepsis, pouch fistula and sexual dysfunction were reported in 7.5% (95%CI: 6.1%-9.1%), 4.5% (95%CI: 3.5%-5.7%) and 3.0% (95%CI: 1.7%-5.2%) of patients. The only complication showing a substantial increase was pouchitis, with a rate of 26.8% (95%CI: 21.0%-33.5%).

Thus it seems that the rate of complications after

IPAA has declined over time (Table 2). The authors of the meta-analyses have noticed that the decline was largest in the earlier period of the IPAA, but seems to have continued over time. Nonetheless, IPAA remains a complex surgery with substantial risk of morbidity. The high rate of pouchitis is also worrisome, since this complication can affect functional outcomes, quality of life and might also increase risk of dysplasia in the pouch. It should be noted that the meta-analyses discussed above did not distinguish between acute and chronic pouchitis, which is an important distinction in terms of course and health implications^[34-36].

Functional outcomes after IPAA were similar in studies published before and after 2000^[33]. Average frequency of bowel movements per 24 h was 5.9 (95%CI: 5.0-6.9), of which 1.5 (95%CI: 1.0-2.1) overnight. Mild and severe faecal incontinence were reported in 14.3% (7.3%-25.9%) and 6.1% (2.9%-12.3%) of patients, respectively. The authors conclude that functional outcomes of IPAA may be determined by an intrinsic limitation of the IPAA procedure, rather than growing expertise or technical refinement. This is in line with other studies showing no improvement in functional outcomes based on technical developments, such as type of anastomosis or laparoscopic approach^[36,37]. However, most patients consider the functional outcome after IPAA to be highly satisfactory, with good quality of life and social functionality that are comparable to those in a healthy reference population^[38,39]. As expected, achieving these adequate quality of life scores was highly correlated with achieving of good functional outcomes^[40].

Cancer risk

The IPAA has as an important advantage the removal of the whole colon and virtually the entire rectum as part of the procedure. This minimizes chances of colon and rectal cancer in this high-risk population. A proctocolectomy should be considered almost mandatory when dysplasia is present. Even when only low-grade dysplasia has been identified by colonoscopy, the risk remains substantial. In such patients, studies show a risk of concomitant cancer or high-grade dysplasia of 15% and a 5-year progression rate of up to 54% if not operated on^[28,41].

When a double-stapled approach for IPAA is used, a mucosal remnant at the anal transition zone (ATZ) is left in place. The risk of cancer in this area is a matter of controversy. In three series with long-term follow-up focused on this outcome, dysplasia and cancer in the anal transitional zone after stapled pouch surgery was found to be infrequent^[7,42,43]. Dysplasia was observed in 8/178

Table 3 Main advantages and disadvantages of ileorectal anastomosis and ileal pouch-anal anastomosis

	IRA	IPAA
Advantages	Easier operation Lower infertility rate Lower risk of urinary and sexual dysfunction Fewer bowel movements per day Better continence	Lower risk of cancer No need for medical therapy Less urgency
Disadvantages	Need for maintenance therapy Risk of recurrent/persistent disease Higher risk of neoplastic degeneration Need for strict surveillance More dietary and work restrictions	Major operation Risk of postoperative complications (pelvic nerves damage, pelvic sepsis, portal vein thrombosis) Pouchitis

IRA: Ileorectal anastomosis; IPAA: Ileal pouch-anal anastomosis.

(4.4%), 7/210 (3.3%) and 0/135 (0%) after at least 10 years of follow-up. In most of these cases, dysplasia developed in the first 2 to 3 years and often disappeared on repeated biopsies. None of the series found cancer in the ATZ after such prolonged follow-up. These data strongly emphasize the extent to which IPAA minimizes the risk of cancer.

The best evidence regarding the development of dysplasia and adenocarcinoma after IPAA can be obtained from a recent study from the Cleveland Clinic^[44], in which 3203 patients undergoing an IPAA from 1984 to 2009 were analyzed. Cumulative incidences for pouch neoplasia at 5, 10, 15, 20, and 25 years were 0.9%, 1.3%, 1.9%, 4.2%, and 5.1%, respectively. Overall, 23 patients (0.72%) developed dysplasia, while 11 (0.36%) developed adenocarcinoma of the pouch and/or the ATZ. Risk factors for pouch neoplasia were also evaluated. Preoperative established cancer [hazard ratios (HR) = 13.43, 95%CI: 3.96-45.53, $P < 0.001$] or dysplasia (HR = 3.62, 95%CI: 1.59-8.23, $P = 0.002$) were the only independent factors associated with increased risk of pouch neoplasia. Mucosectomy did not protect against this risk, and the rate of pouch cancer was actually higher after mucosectomy with a rate of 1.3% (6/451) compared to 0.3% (9/2734) after the double-stapled approach. The authors^[44] concluded that the risk for neoplasia in patients with UC and IPAA is small, and that it is mainly determined by the presence of preoperative dysplasia or cancer.

Additionally, in a review of literature, 26 published case reports were identified between 1984 and 2008^[45]. Certain observations from this review are noteworthy. First, of the 26 carcinomas, 14 (52%) arose from rectal mucosa or from the anal transition zone, while 6 (23%) were from ileal pouch mucosa. Second, adenocarcinomas developed after mucosectomy in 17 patients, and after a double-stapled approach in 8 patients (1 case not reported). Also worth noting, the indication for the IPAA was due to neoplasia in 19 patients (9 cancers and 10 dysplasia) and non-neoplasia in 6 patients. The median time for development of pouch lesions was the shortest in patients operated on for cancer (median 3 years), compared to a median of 6.5 in the other patients. This review is in line with results from the above mentioned study, and

further establishes the following conclusions: (1) the low number of reported cases; (2) cancer can develop both after mucosectomy or double-stapled approach; and (3) the close relationship between surgery for neoplasia and development of cancer. The review was not able to estimate the incidence of cancer after IPAA, since the total number of IPAA cases was not stated in most case reports. Branco *et al*^[45] did publish their own case as part of this review, which was the first case they observed in a cohort of 520 patients (0.2%) from 1978 to 2008. This percentage is also in line with the Cleveland study^[44].

Despite this seemingly small risk, surveillance of selected patients has been recommended by some authors^[46,47]. This approach might especially be important in UC patients with dysplasia or cancer present at time of surgery, or patients with retained rectal mucosa and active inflammation (*i.e.*, cuffitis). Also the presence of chronic pouchitis might be a valid indication for surveillance, since this has been associated with increased risk of low-grade dysplasia (odds ratio 13.48, $P < 0.02$), as well as high-grade dysplasia (3/66 *vs* 0/210, $P = 0.01$)^[35].

CONCLUSION

In the current era IPAA is the preferred approach for patients with UC requiring surgical treatment. The removal of all diseased mucosa and the lower risk of cancer after IPAA compared to IRA are the main advantages of this technique (Table 3). Therefore, IPAA should certainly be performed when the rectum is actively involved in the disease or when dysplasia or cancer are present in any part of the colon or rectum. Nonetheless, there is still a role for IRA and TPC for selected patients and for patients not candidates for IPAA.

Total abdominal colectomy with IRA is justified in UC patients with normal anal sphincters tone without severe perineal disease, and spared and distensible rectum with no evidence of dysplasia or cancer at the time of intervention. It can be also proposed to young women as a possible interim procedure based on concerns for infertility after IPAA.

The risk of cancer is of particular concern in the comparison between these two techniques. Current evi-

Table 4 Risk of cancer after ileorectal vs ileal pouch-anal anastomosis in ulcerative colitis

	Period	n	Follow-up average (yr)	Overall cancer rate (%)	Estimated cumulative risk after 20 years (%)
Ileorectal anastomosis					
da Luz Moreira <i>et al</i> ^[11]	1971-2006	86	9	8	14
Leijonmarck <i>et al</i> ^[13]	1955-1984	51	13	0	-
Pastore <i>et al</i> ^[15]	1974-1990	48	6.3	2	14.3 ¹
Börjesson <i>et al</i> ^[16]	1997-2003	32	3.5	0	-
Grundfest <i>et al</i> ^[17]	1957-1977	89	8	4.8	5 ± 3.5
Elton <i>et al</i> ^[18]	1990-1999	18	2.6	-	-
Andersson <i>et al</i> ^[19]	1992-2006	105	5.4	-	2.1
Lepistö <i>et al</i> ^[22]	1978-2000	20	18	0	-
Oakley <i>et al</i> ^[23]	1960-1982	288	8.2	3.1	-
Baker <i>et al</i> ^[26]	1952-1976	374	> 10	5.9	6 ± 2
Ileo-pouch anal anastomosis					
Kariv <i>et al</i> ^[44]	1984-2009	3203	± 12	0.4	4
Branco <i>et al</i> ^[45]	1978-2008	520	± 15	0.2	-

¹Cumulative risk at 12 years (rather than 20).

dence shows a large variation in the reported rates of cancer after IRA from 0% to 8%. For IPAA, this risk is much smaller, and two large series have shown a rate of cancer of about 0.3%. Few studies have calculated the cumulative risk of cancer as well. Similarly, estimated cumulative risk of cancer after 20 years was higher after IRA (6% to 14%) compared to IPAA (4.2%) (Table 4).

Therefore, every patient undergoing IRA should be informed about the risk of recurrent proctitis and cancer in long standing disease. They have to fully understand the need for meticulous surveillance and agree to comply with at least yearly endoscopy with rectal biopsies. Unless these conditions are met, patients should not be offered an IRA. Also, patients with widely metastatic colorectal cancer may benefit from an IRA as a palliative procedure.

Functional results seem to be better after IRA with lower frequency of bowel movements and less night-time seepage but with more urgency compared to patients with an IPAA. The overall quality of life is similar, although the IRA group has significantly more dietary and work restrictions^[11].

Finally, TPC still remains the procedure of choice in patients with impaired anal sphincter function and high-risk of pouch failure.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Crohn's disease and growth deficiency in children and adolescents

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of behavior may cause growth deficiencies (in weight and height) and delayed puberty, with several studies reporting that these patients may not reach an optimal bone mass. Glucocorticoids and inflammation inhibit bone formation, though their impact on skeletal modeling remains unclear. Long-term control of active inflammation and an adequate intake of nutrients are both fundamental in promoting normal puberty. Recent evidence suggests that recombinant growth factor therapy is effective in improving short-term linear growth in selected patients, but is of limited benefit for ameliorating mucosal disease and reducing clinical disease activity. The authors conclude that an intense initial treatment (taking a "top-down" approach, with the early introduction of immunomodulatory treatment) may be justified to induce and maintain remission so that the growth of children with CD can catch up, ideally before puberty. Exclusive enteral nutrition has a key role in inducing remission and improving patients' nutritional status.

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Abstract

Nutritional concerns, linear growth deficiency, and delayed puberty are currently detected in up to 85% of patients with Crohn's disease (CD) diagnosed at childhood. To provide advice on how to assess and manage nutritional concerns in these patients, a Medline search was conducted using "pediatric inflammatory bowel disease", "pediatric Crohn's disease", "linear growth", "pubertal growth", "bone health", and "vitamin D" as key words. Clinical trials, systematic reviews, and meta-analyses published between 2008 and 2013 were selected to produce this narrative review. Studies referring to earlier periods were also considered if the data was relevant to our review. Although current treatment strategies for CD that include anti-tumor necrosis factor- α therapy have been shown to improve patients' growth rate, linear growth deficiencies are still common. In pediatric CD patients, prolonged diagnostic delay, high initial activity index, and stricturing/penetrating type

Key words: Bone health; Enteral nutrition; Growth; Height; Pediatric inflammatory bowel disease; Pediatric Crohn's disease; Linear growth; Pubertal growth; Vitamin D; Weight loss

Core tip: This review focuses on current evidence for managing growth issues in children diagnosed with Crohn's disease. Long-term control of active inflammation and an adequate intake of nutrients are both essential in promoting puberty. Exclusive enteral nutrition has a key role, as it induces disease remission and improves nutritional status. The early introduction of immunosuppressants or biologics may be justified in children to achieve disease remission and enable their growth to catch up, ideally before puberty. Recent evidence suggests that recombinant growth factor therapy is effective in improving short-term linear growth.

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INTRODUCTION

Crohn's disease (CD) is a global health concern and a condition that significantly affects patients' quality of life, as well as placing a heavy financial burden on the community^[1]. CD is currently without a cure, and its incidence is rising not only in Western countries, but also in most developing countries. It manifests in childhood or adolescence in up to 25% of cases^[2].

The microbial ecosystem colonizing the human bowel is influenced by diet, which prompts metabolic processes essential to bowel metabolism^[3-7]. Genetic susceptibility, intestinal microbiota, lifestyle and environmental factors are amongst the potential mechanisms involved in the pathogenesis of inflammatory bowel diseases (IBD)^[3]. Prolonged diagnostic delays, high initial activity indexes, and stricturing/penetrating behavior patterns may predict subsequent complications and the need for surgery, thus justifying a resort to early intensive therapy. The early introduction of immunomodulatory therapy favorably affects the course of IBD^[8-12]. Growth failure and impaired nutritional status are seen in 65%-85% of children and adolescents diagnosed with CD, and 15%-40% of these patients continue to suffer from growth deficiency throughout the course of their disease^[1,13]. Exclusive enteral nutrition has become a key treatment strategy for inducing disease remission in pediatric CD, offering the advantage of improving patients' nutritional status as well as enabling the mucosa to heal at much the same rate as is achievable with corticosteroids^[1,14-17].

GROWTH ISSUES IN PEDIATRIC CROHN'S DISEASE

Growth deficiency can severely affect quality of life for children and adolescents with CD, and complicate their management^[18-20]. It occurs in a significant proportion of patients (up to 85%), and may even precede any clinical evidence of bowel disease. Abraham *et al*^[21] recently conducted a systematic review focusing on understanding the long-term risks of growth deficiency, disease reclassification and extension, hospitalization, cancer, and death among patients with childhood IBD^[21] (Table 1): in 41 studies considered, concerning 3505 patients with CD, 2071 with ulcerative colitis (UC) and 461 with non-IBD colitis, growth failure was identified more often in CD (10%-56%) than in UC (0%-10%) or non-IBD controls. Growth improved after surgical resection in patients with CD^[21].

Among IBD sufferers, male patients are more vulner-

able to growth deficiencies than females of the same age because the male growth spurt in puberty is greater, occurs later, and lasts longer than in females^[22]. Vasseur *et al*^[23] examined a total of 261 pediatric patients with CD registered in the EPIMAD registry in northern France. At diagnosis, 25 children (9.5%) had a height more than 2 standard deviations below the norm, and the same applied to the weight of 70 children (27%), and the BMI of 84 children (32%). At maximal follow-up, 18 children (6.9%) had a growth deficiency and 40 (15%) suffered from malnutrition. Nutritional status was more severely impaired in children with stricturing disease. Growth and nutritional deficiency at diagnosis, young age, male gender, and extraintestinal manifestations at diagnosis were indicators of a poor prognosis. The authors concluded that young boys with substantial inflammatory manifestations of CD are at higher risk of subsequent growth failure, especially when their growth is already deficient at diagnosis^[23].

Assessing growth in IBD patients of a developmental age is so important that it was included among the key points in the Paris classification of pediatric CD, which replaced the previous Montreal classification^[24]. The following factors are implicit in the physiopathology of growth deficiency in pediatric IBD^[22]: (1) chronic calorie insufficiency: these patients' malnutrition is due to a lower intake, protein malabsorption, abnormal intestinal losses, and anorexia correlating with the pathological picture [tumor necrosis factor- α (TNF- α) also has a direct influence at the hypothalamic level]. Inflammatory mediators trigger an increase in basal metabolism too, coinciding with a further deterioration in nutritional status; (2) direct cytokine effects: insulin-like growth factor (IGF)-1 is produced in the liver and is the principal mediator of the effects of growth hormone (GH). Patients suffering from CD have significantly reduced IGF-1 blood levels, irrespective of their GH levels. TNF- α and interleukin (IL)-6 also have a direct inhibitory effect on GH. Other pathways independent of IGF-1 inhibition by means of which the inflammatory cytokines inhibit the linear growth rate have recently been identified as well; (3) effects of chronic treatment with corticosteroids: these drugs induce a central suppression of GH production and reduce IGF-1 synthesis in the liver, as well as interfering with its peripheral receptor activity; (4) effects of IBD on endocrine growth mediators: delayed puberty gives rise to a sex hormone deficiency that may be involved in growth deficiencies; and (5) genetic factors: polymorphisms of NOD2/CARD15 and other already-identified genes appear to generate a cytokine pattern capable of contributing to pediatric IBD patients' growth deficiency; promoter regions of the gene coding for TNF- α and IL-6 also seem to be involved.

NUTRITIONAL CONCERNS IN PEDIATRIC CROHN'S DISEASE

Nutritional issues are often associated with CD, especially in pediatric cases, with underweight and stunting

Table 1 Summary of the main studies that were reviewed on nutritional concerns in pediatric Crohn's disease

Ref.	Type of study	Patients	Results	Conclusion
Vaisman <i>et al</i> ^[25] , <i>Nutrition</i> 2006	Prospective cohort study	16 pts with CD; Age 19-57 yr Remission of disease (CDAI Activity Disease Index < 150); 2 groups (BMI 18.5 kg/m ² as a cutoff point)	Subjects with lower BMIs tended to have less lean body mass ($P = 0.006$), less bone mineral density ($P = 0.006$), and lower resting energy expenditure ($P = 0.003$); No correlation between BMI and energy intake, although percentage of malabsorption negatively correlated with BMI ($P = 0.07$)	In the presence of similar energy intake, resting energy expenditure does not seem to contribute to lower BMI, although nutrient malabsorption is higher in malnourished patients with CD in remission; Malabsorption should be evaluated in patients with CD who fail to gain Wt during disease remission, to establish their extra caloric requirements
Gupta <i>et al</i> ^[28] , <i>Inflamm Bowel Dis</i> 2013	Retrospective review	43 IBD pts (mean age 12.8 yr; range 5.1-17.4 yr) 67% M 33% F	Reductions in erythrocyte sedimentation rate ($P < 0.0001$) and C-reactive protein ($P < 0.02$), and increases in albumin ($P < 0.03$); Mean PCDAI score 26.9 at baseline and 10, 2 at follow-up ($P < 0.0001$); Induction of remission achieved in 65% and response in 87% at a mean follow-up of 2 mo (1-4 mo)	Novel protocol for enteral nutrition (80%-90% of patient's caloric needs) seems to be effective for the induction of remission in CD children; The protocol may result in improved EN acceptance and compliance and will be evaluated prospectively
Wiskin <i>et al</i> ^[29] , <i>J Hum Nutr Diet</i> 2012	Prospective cohort study	46 IBD children	No children scored low risk with STAMP, STRONGkids or PNRS; 23 children scored low risk with PYMS; Good agreement between STAMP, STRONGkids, and PNRS ($K > 0.6$); Modest agreement between PYMS and the other scores ($K = 0.3$); No agreement between the risk tools and the degree of malnutrition based on anthropometric data ($K < 0.1$)	Relevance of nutrition screening tools for children with chronic disease is unclear; There is the potential to under recognize nutritional impairment (and therefore nutritional risk) in children with IBD
Valentini <i>et al</i> ^[30] , <i>Nutrition</i> 2008	Prospective, controlled, multicentric study	94 pts with CD (CDAI 71 +/- 47) 61 F 33 M 50 UC (UCAI 3.1 +/- 1.5) 33 F 17 M 61 healthy control subjects 41 F 20 M from centers in Berlin (Germany), Vienna (Austria), and Bari (Italy) 47 well-nourished patients with IBD pair-matched to healthy controls by BMI, sex, and age	74% IBD patients were well-nourished according to the SGA, BMI, and serum albumin; Body composition analysis demonstrated a decrease in BCM in patients with CD ($P = 0.021$) and UC ($P = 0.041$) compared with controls; Handgrip strength correlated with BCM ($r = 0.703$, $P = 0.001$) and was decreased in patients with CD ($P = 0.005$) and UC ($P = 0.001$) compared with controls; Lower BMC in patients with moderately increased serum CRP levels compared with patients with normal levels	In CD and UC, selected micronutrient deficits and loss of BCM and muscle strength are frequent in remission and cannot be detected by standard malnutrition screening
Chan <i>et al</i> ^[31] , <i>Am J Gastroenterol</i> 2013	Prospective cohort study	300724 participants (recruited into the European Prospective Investigation into Cancer and Nutrition study) 177 UC and 75 CD	No associations with the four higher categories of BMI compared with a normal BMI for UC (P trend = 0.36) or CD (P trend = 0.83); Lack of associations when BMI analyzed as a continuous or binary variable (BMI 18.5 kg/m ² vs ≥ 25 kg/m ²); Physical activity and total energy intake not associated with UC (P trends 0.79-0.18) or CD (P trends 0.42-0.11)	Obesity as measured by BMI not associated with the development of incident UC or CD; Alternative measures of obesity required to further investigate the role of obesity in the development of incident IBD
Werkstetter <i>et al</i> ^[32] , <i>J Crohns Colitis</i> 2012	Prospective cohort study	39 IBD children in remission; 27 CD, 12 UC 24 M; 39 healthy age-sex-matched controls	IBD pts vs controls: Lower Z-scores for phase angle α [-0.72; 95%CI: (-1.10-0.34)] Lower grip strength [-1.02 (-1.58-0.47)] Lesser number of steps per day [-1339 (-2760-83)] Shorter duration of physical activity [-0.44 h (-0.94-0.06)], particularly in F and patients with mild disease. Quality of life and energy intake did not differ between patients and controls	In spite of quiescent IBD, lean body mass and physical activity were reduced; Interventions to encourage physical activity may be beneficial in this lifelong disease

Gerasimidis <i>et al</i> ^[33] <i>Inflamm Bowel Dis</i> 2013	Prospective cohort study	184 new pediatric IBD Dg 139 one year follow-up IBD children 84 children treated with EEN	72% anemic at Dg; Anemic children with CD had shorter diagnosis delay, lower BMI, lower Dg delay ($P < 0.001$) and BMI Z-score, $P = 0.003$) than non-anemic patients; Extensive colitis associated with severe anemia in UC; After EEN, severe anemia decreased (32%-9%, $P < 0.001$) and hemoglobin concentration increased by 0.75 g/dL	Anemia is frequent at Dg and follow-up and should receive more attention from the clinical team; The focus should remain suppression of inflammatory process in active disease
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Dg: Diagnosis; pt/pts: Patient/patients; CD: Crohn's disease; UC: Ulcerative colitis; IBD-U: Unclassified IBD; rhGH: Recombinant growth factor; ESR: Erythrocyte sedimentation rate; BMC: Body cell mass; BMI: Body mass index; Ht: Height; Wt: Weight; BMD: Bone mineral density; DXA: Dual-energy X-ray absorptiometry; EEN: Exclusive enteral nutrition; GH: Growth hormone; (P)CDAI: (Pediatric) Crohn's disease activity index; PUCAI: Pediatric ulcerative colitis activity index; M: Male; F: Female.

commonly seen at presentation, as well as linear growth retardation and delayed puberty developing later on^[1]. Undernutrition has been reported in 65%-75% of patients with CD^[25] (Table 1), and recent weight loss is one of the triad of clinical manifestations of the disease. Although medical treatment can soon restore body weight, this is not reflected in concomitant changes in body composition. Children with CD have the features of nutritional cachexia, with normal fat stores but depleted lean mass. Poor bone health, delayed puberty, and growth failure are other possible features complicating their clinical management^[26].

As growth impairment is mainly secondary to disease activity, all available pharmacological steps to induce remission in a given patient (depending on their disease phenotype) should have a positive effect on growth as well. Exclusive enteral nutrition has been used as a therapeutic approach to CD because it can improve patients' nutritional status and induce remission (mucosal healing) as quickly as corticosteroids^[1,27]. Exclusive enteral nutrition has thus become a fundamental option at many centers treating pediatric CD^[1]. A recent retrospective review by Gupta *et al*^[28] (Table 1) assessed the efficacy of enteral nutrition (EN) in delivering 80%-90% of patients' calorie needs with a view to inducing remission in pediatric patients with CD. This approach allowed for patients to ingest the remainder of the calories they needed from a normal diet, and so it differs from the standard practice of providing EN to cover 100% of patients' calorie needs. The sample's mean Pediatric Crohn's Disease Activity Index score (PCDAI) at the baseline was 26.9 and it dropped to 10.2 at follow-up ($P = 0.0001$). Remission was induced in 65% of cases and response in 87% after a mean 2 mo of follow-up (1-4 mo). The authors concluded that this novel EN protocol seems to be effective in inducing remission in pediatric patients with CD, helping to increase their weight and improve their laboratory markers. This protocol may also make EN more readily acceptable to patients and improve their compliance^[28].

There has recently been increasing interest in the use of nutrition risk assessment tools in children to identify those needing nutritional support^[29] (Table 1). Four screening tools that are not disease-specific [the Screening tool for the assessment of malnutrition in pediatrics

(STAMP), the screening tool for risk on nutritional status and growth (STRONGkids), the pediatric Yorkhill malnutrition score (PYMS), and the simple pediatric nutrition risk score (PNRS)] were applied by Wiskin *et al*^[29] to 46 children with IBD. The degree of malnutrition was measured by anthropometry alone using the World Health Organization's International Classification of Diseases (ICD-10) criteria. There was a good agreement between STAMP, STRONGkids, and PNRS ($K > 0.6$), but only a modest agreement between PYMS and the other scores ($K = 0.3$), and no agreement between the risk tools and the degree of malnutrition based on anthropometric data ($K < 0.1$). The authors concluded that the relevance of nutrition screening tools for children with chronic intestinal disease is unclear, and there is a risk of their failing to recognize nutritional impairment (and consequent nutritional risk) in children with IBD^[29].

A study by Vaisman *et al*^[25] (Table 1) focused on identifying the relative contribution of factors causing malnutrition in a sample of 16 patients with CD in remission (age 19-57 years). Resting energy expenditure (REE) was studied by indirect calorimetry and body composition by dual-energy X-ray absorptiometry. Subjects with lower BMIs tended to have less lean body mass ($P = 0.006$), a lower bone mineral density ($P = 0.006$), and lower REE ($P = 0.003$). No correlation emerged between BMI and energy intake, although the percentage of malabsorption correlated negatively with BMI ($P = 0.07$). The authors concluded that nutrient malabsorption is more severe in malnourished patients with CD in remission, and consequently suggested that malabsorption should be assessed in CD patients who fail to gain weight while in remission in order to establish their extra calorie needs^[25].

A prospective, controlled, multicentric study by Valentini *et al*^[30] (Table 1) considered nutritional status (subjective global assessment [SGA], BMI, albumin, trace elements), body composition (bioelectrical impedance analysis, anthropometry), muscle strength, and quality of life in 94 patients with CD and 50 with UC, all in clinical remission, and 61 healthy controls. Most patients with IBD (74%) were well-nourished according to their SGA, BMI, and serum albumin levels, but body composition analysis demonstrated a lower body cell mass (BCM) in patients with CD ($P = 0.021$) and UC ($P = 0.041$) than in

controls. Handgrip strength correlated with BCM ($P = 0.001$) and was again lower in patients with CD ($P = 0.005$) and UC ($P = 0.001$) than in controls. These differences were seen even in patients classified as well-nourished. BCM was lower in patients with moderately increased serum C-reactive protein levels than in patients with normal levels. The authors concluded that selected micronutrient deficits and loss of BCM and muscle strength are frequent in CD and UC in remission, and go undetected in standard malnutrition screening^[30].

Obesity is associated with a pro-inflammatory state that may be involved in the etiology of IBD. Chan *et al*^[31] (Table 1) conducted the first prospective cohort study to identify any association between obesity and the onset of incident IBD in a sample of 300724 participants recruited for the European Prospective Investigation into Cancer and Nutrition study. At recruitment, anthropometric measurements were taken of patients' height and weight, and their physical activity and total energy intake were recorded using validated questionnaires. The cohort was monitored and 177 participants developed incident UC, while 75 developed incident CD. No associations emerged vis-à-vis UC or CD between the four higher BMI categories and a normal BMI level. Physical activity and total energy intake (factors influencing BMI) also revealed no association with UC or CD. The authors concluded that obesity, as measured by the BMI, is unassociated with the onset of incident UC or CD. Alternative obesity measures are needed to further clarify the role of obesity in the onset of incident IBD^[31].

Physical activity is important for muscle and bone strength in growing children and may be limited in pediatric IBD patients, even when their disease is quiescent^[32]. A recent study by Werkstetter *et al*^[32] (Table 1) compared 39 IBD patients (27 CD, 12 UC) in remission (or with only mild disease activity) with 39 healthy age- and sex-matched controls. The patients had lower Z-scores for phase angle α and lower handgrip strength than controls. They tended to take fewer steps per day and engage in shorter periods of physical activity, particularly among females and patients with mild disease. The authors concluded that, even with quiescent disease, IBD patients have reduced levels of lean body mass and physical activity. Action to encourage them to engage in physical exercise may therefore be beneficial in this lifelong disease^[32].

Low concentrations of plasma micronutrients are commonly reported in IBD patients, but may be difficult to interpret in the presence of an acute phase response, and other body store adequacy indices are needed. Anemia is a common extraintestinal manifestation in IBD children: these are primarily cases of iron-deficiency anemia, with anemia of chronic disease coming second^[33]. A study by Gerasimidis *et al*^[33] (Table 1) explored the epidemiology of anemia and associated factors in children with IBD at the time of their diagnosis, after 1 year, and during treatment with exclusive enteral nutrition (EEN). At diagnosis, 72% of the children were anemic. Children with CD who were anemic had a shorter diagnostic delay

and a lower BMI than those who were not ($P = 0.003$). Extensive colitis was associated with severe anemia in UC. After EEN, the cases of severe anemia decreased (32%-9%; $P = 0.001$), and hemoglobin concentrations increased by 0.75 g/dL. The authors concluded that children with IBD are highly likely to have anemia at diagnosis, and that this matter should receive more attention during their follow-up, even though clinicians should focus on suppressing the inflammatory process in cases of active disease^[33].

Deficiencies in the liposoluble vitamins A-D and E, and zinc are also possible features of IBD patients^[34-36].

Vitamin D

Vitamin D is a key factor not only for its role in the mineralization of bone and teeth, but also because of its other metabolic functions and its protective role in immune-mediated diseases and allergies^[37-39]. Vitamin D status is assessed by testing its metabolite 25-hydroxy-vitamin D (25-OH-D) in plasma or serum, which reflects the amount of vitamin D converted in the skin through sunlight exposure and ingested in the diet^[37]. Poor vitamin D status may have detrimental consequences for the future health of a child, so an optimal vitamin D status is a crucial public health goal. Vitamin D levels are classed as severely deficient for levels < 37 nmol/L, insufficient for levels < 50 nmol/L, and suboptimal for levels of 50-75 nmol/L^[37]. Exposure to sunlight is generally the most important source of vitamin D3, while the contribution of vitamin D from foods and supplements is fundamental in populations living at latitudes with limited hours of sunlight^[37]. Obesity is a risk factor for vitamin D deficiency because a greater proportion of the body's vitamin D remains stored in adipose tissue^[37].

Current national recommendations suggest a daily intake of 7.5 mcg of vitamin D^[37]. Foods containing large amounts of vitamin D include oily fish and eggs. Vitamin D is produced endogenously in the skin by the photo-reduction of 7-dehydrocholesterol by ultraviolet light. Human exposure to sunlight is limited throughout their lives and some foods are fortified with vitamin D (*e.g.*, milk, some juices, breads, and cereals). Children with chronic diseases are consequently at risk of vitamin D deficiency. The Institute of Medicine recommends a vitamin D intake of 600 IU/d in individuals 1-70 years of age, plus 700-1300 mg/d of calcium (depending on age) to promote healthy skeletal growth^[40]. Vitamin and mineral deficiencies have been described in patients with IBD and are attributed to gut mucosa inflammation and a reduced oral intake^[34]. Cranney *et al*^[41] conducted a systematic qualitative review of 167 eligible studies (112 randomized controlled trials (RCTs), 19 prospective cohort studies, 30 case-control, and 6 before-after studies). The largest body of evidence on vitamin D status and bone health concerned older adults, while few studies focused on infants, children, and adolescents. There was inconsistent evidence of an association between circulating 25(OH)D levels and bone mineral content in infants. In adoles-

cents, there was a fair amount of evidence for an association between 25(OH)D levels and changes in BMD. There was solid evidence of the use of foods fortified with vitamin D (11 RCTs) consistently increasing serum 25(OH)D in both young and older adults. In short, the studies generated fairly good evidence of an association between circulating 25(OH)D concentrations and some bone health outcomes (established rickets, PTH, falls, BMD). When compared with a placebo, vitamin D(3) (> 700 IU/d) with calcium supplementation reportedly had a small beneficial effect on BMD and reduced the risk of fractures and falls, although this benefit may be confined to specific subgroups^[42]. A recent retrospective study performed by Alkhouri *et al.*^[34] in the US investigated the prevalence of vitamin and zinc deficiencies in 61 children (age 1-18 years) with newly-diagnosed IBD from 2006 to 2010 (80% with ileal inflammation) by comparison with a control group of 61 age- and sex-matched individuals. While none of the IBD patients had folate or vitamin B12 deficiency, 62% of them had vitamin D deficiency (*vs* 75% in the control group), 16% had vitamin A deficiency, 5% had vitamin E deficiency (*vs* 8% in the control group), and 40% had zinc deficiency (*vs* 19% in the control group). The authors concluded that vitamin B12 and folate deficiencies are rare in children with newly-diagnosed IBD in the United States, so there is no reason to support their routine monitoring. On the other hand, vitamin A and zinc deficiencies were statistically more prevalent among the IBD cases than in controls, so their levels should be assessed at the time of their diagnosis to enable enteral supplementation to be started^[34]. Vitamin D deficiency was common in the population tested, so routine screening for this deficiency and supplementation are warranted. These results contrast with previous studies by Yakut *et al.*^[43] and Chowers *et al.*^[44].

To sum up on vitamin D, there is still no strong evidence in the literature of an association between circulating 25(OH)D concentrations and bone health outcomes, and an improvement in BMD in particular. Only partial benefits of administering vitamin D(3) (> 700 IU/d) and calcium supplementation have been seen in terms of BMD and a lower risk of fractures and falls, and only in specific subgroups^[41]. Supplementing vitamin D in pediatric patients is nonetheless generally recommended when its levels are found to be depleted, given its action not only on bone metabolism but also in terms of immunomodulation^[37,40].

BONE HEALTH AND PEDIATRIC CROHN'S DISEASE

Although current treatment strategies for CD that include therapy against TNF- α have been found to speed up growth, linear growth deficiencies persist even with optimized therapy^[22]. Children with CD continue to suffer from short stature and slow growth, and several studies have indicated that children with IBD may fail to achieve optimal bone mass^[45-47]. Children with CD have multiple

risk factors for impaired bone accrual^[48]. The skeleton is a highly dynamic tissue regulated by local, systemic, and environmental clues that modify osteoblastic (bone formation) and/or osteoclastic (bone reabsorption) activities. IBD affects bone regulation at all levels: environmentally through intestinal barrier breaks and/or a microbial composition in the gut; systemically with the circulation of gut immune cells and cytokines throughout the body; and locally by causing inflammation of extra-intestinal organs (such as the bone marrow)^[49].

Bone formation and reabsorption are significantly involved in bone health and growth. In children with CD, both of these processes are impaired, so bone growth is ultimately suboptimal^[49]. Factors contributing to this derangement are inflammation, delayed growth and puberty, lean mass deficiencies, and the use of glucocorticoids^[50,51]. A recent study by Irwin *et al.*^[52] examined the effect of experimental IBD on bone health. Interleukin-10-deficient animals infected with *Helicobacter hepaticus* (*H. hepaticus*) were used as a murine model of colitis, and the molecular and histological properties of their bone and intestine were examined to identify the immunopathological consequences of colitis in mice. Six weeks after they were infected, male (but not female) mice revealed significant trabecular bone loss in the distal femur and vertebrae. The authors concluded that the severity of *H. hepaticus*-induced colitis and the associated bone loss are gender-related, possibly as a result of gender-specific effects on *H. hepaticus* colonization in the mouse gastrointestinal tract and the consequent immunopathological responses^[52].

A prospective study by Kim *et al.*^[45] (Table 2) aimed to examine the risk factors and extent of bone mass reduction and to analyze the impact of IBD developing early, before bone mass has peaked (*i.e.*, before the maximal bone mineral density has been reached during development). Bone mineral density (BMD) was assessed in the lumbar spine and hip bone in 44 IBD patients (21 of them < 30 years old). Younger patients had a significantly more severe bone mass reduction in the lumbar spine than patients aged > 30 years (multivariate analysis showed a hazard ratio of 3.96, $P = 0.06$)^[45]. On the other hand, a recent prospective cohort study by Tsampalieros *et al.*^[53] (Table 2) suggested that younger age provides a window of opportunity for skeletal recovery. The aim of their study was to examine changes in BMD and cortical structure after CD had been diagnosed, and to identify associations with growth, glucocorticoids, and disease activity. The authors concluded that CD was associated with a persistently low trabecular BMD, although younger participants showed a greater potential for recovery. A greater linear growth was associated with a greater recovery of cortical dimensions, especially among participants with less glucocorticoid exposure and inflammation. So, although glucocorticoids and inflammation inhibit bone formation, their impact on skeletal modeling is still not clear^[53].

Another longitudinal study performed by Schmidt *et al.*^[51] (Table 2) on a total of 144 patients with IBD (including 83 with UC and 45 with CD) concluded that IBD

Table 2 Summary of the main studies that were reviewed on growth issues and bone health in pediatric Crohn's disease

Ref.	Type of study	Patients	Results	Conclusion
Abraham <i>et al</i> ^[21] <i>J Clin Gastroenterol</i> 2012	Systematic review	3505 CD, 2071 UC, and 461 IBD-U (age at onset < 18 yr)	Growth failure was reported in CD (10% and 56%) more often than UC (0%-10%) or non-IBD controls; Improvements in growth occurred after surgical resection in CD pts; Increase in disease reclassification over time from UC and IBD-U Dg to CD; CD pts had higher number of hospitalizations and hospital days per year <i>vs</i> UC pts in most studies; The reported surgery rates in CD ranged between 10% and 72%; the colectomy rates in UC ranged between 0% and 50%	Childhood-onset IBD pts had growth failure reported in pts with CD more often than those with UC, and had a reclassification of disease type to CD over time; Higher rates of surgery and hospitalizations were found with CD than with UC
Kim <i>et al</i> ^[45] <i>Clin Endosc</i> 2013	Prospective cohort study	44 IBD (21 aged < 30 yr; 23 aged > 30 yr)	Significant bone mass reduction at the LS in IBD patients aged < 30 yr <i>vs</i> patients aged > 30 yr (BMD $P < 0.01$; T-score $P < 0.01$; Z-score $P < 0.01$); Multivariate analysis: risk factor of bone mass reduction for patients < 30 yr \rightarrow HR = 3.96, $P = 0.06$	Bone mass reduction is more severe in patients diagnosed with IBD before the age of 30 yr
Schmidt <i>et al</i> ^[51] <i>J Pediatr Gastroenterol Nutr</i> 2012	Longitudinal cohort study	144 IBD pts (83 UC, 45 CD)	Children with UC and CD had significantly lower mean BMD Z-scores for the LS at baseline and after 2 yr; The reduction in BMD was equally pronounced in patients with UC and CD; Neither group improved their Z-score during the follow-up period; Significantly lower mean BMD Z-scores for the LS were found at baseline in M ($P < 0.001$), but not in F; Lowest BMD values in the group of patients ages 17 to 19 yr in M and in F	The entire group of pediatric patients with IBD showed permanent decreases in their BMD Z-scores for the LS; however, afflicted children have the potential to improve their BMD by the time they reach early adulthood
Tsampalieros <i>et al</i> ^[53] <i>J Clin Endocrinol Metab</i> 2013	Prospective cohort study	CD (age 5-21)	Disease activity improved over the study interval ($P < 0.001$); Trabecular BMD-Z improved over the first 6 mo; Increases associated with improved disease activity ($P < 0.001$), younger age ($P = 0.005$), and increases in vitamin D levels ($P = 0.02$); Greater increases in tibia length associated with greater increases in cortical area-Z ($P < 0.001$); Greater glucocorticoid doses and disease activity significantly associated with failure to accrue cortical area, and more pronounced with greater linear growth (interaction $P < 0.05$); Mean \pm SD trabecular BMD and cortical area Z-scores significantly reduced at the final visit	CD was associated with persistent deficits in trabecular BMD; Younger participants demonstrated greater potential for recovery; Greater linear growth associated with greater recovery of cortical dimensions, especially among participants with lesser glucocorticoid exposure and inflammation; Younger age and concurrent growth provide a window of opportunity for skeletal recovery
Malik <i>et al</i> ^[54] <i>J Crohns Colitis</i> 2012	Prospective cohort study	36 children with CD (Male 22)	28 (78%) CD children treated with adalimumab went into remission; Overall 42% of children showed catch-up growth, which was more likely in: Pts who achieved remission ($P = 0.007$); Pts who were on immunosuppression ($P = 0.03$); Pts whose indication for adalimumab was an allergic reaction to infliximab ($P = 0.02$); Pts who were on prednisolone when starting adalimumab, ($P = 0.04$)	Clinical response to adalimumab is associated with an improvement in linear growth in a proportion of children with CD; Improved growth is more likely in patients entering remission and on immunosuppression but is not solely due to a steroid sparing effect
Malik <i>et al</i> ^[55] <i>Arch Dis Child</i> 2012	Retrospective cohort study	116 CD children; 68 M; Mean age at diagnosis 10.8 yr (range 4.9-15.5); Mean age at maximum follow-up of 15.4 yr (9.4-19.3)	At T0, mean height SD score was -0.5 (-3.3-2.6) compared to a mid-parental mean height SD score of 0.2 (-2.0-1.4) ($P = 0.002$); At T1, T2, T3, and maximum follow-up, mean height SD score was -0.6 (-4.8-7.8), -0.6 (-2.9-2.2), -0.7 (-3.6- 2.5) and -0.5 (-3.5-2.9), respectively; Mean Ht velocity SDS at T1, T2, T3 and maximum follow-up was -1.4 (-7.4-7.4), -0.6 (-7.5-6.1), -0.1 (-6.6 -7.6) and 0.6 (-4.8-7.8), respectively ($P < 0.05$)	In final models: Mean Ht velocity SDS was associated negatively with the use of prednisolone ($P = 0.0001$), azathioprine ($P = 0.0001$), methotrexate ($P = 0.0001$), and weight SDS (WtSDS) $P = 0.0001$; Mean Ht velocity SDS was associated positively with age ($P = 0.0001$) and Wt SDS ($P = 0.01$); Δ Ht SDS was associated negatively with use of prednisolone ($P < 0.02$)

Laakso <i>et al</i> ^[56] <i>Calcif Tissue Int</i> 2012	Cross-sectional Cohort Study	80 IBD pts (median age 14.9 yr, range 5-20), median disease duration 3.4 yr; 51 UC, 26 CD, and 3 IBD-U	IBD pts had lower bone age-adjusted LS and whole-body areal BMD ($P < 0.001$ for both) and whole-body composition adjusted for Ht ($P = 0.02$) than controls; Lean mass and fat mass Z-scores did not differ between the groups, but IBD patients had lower whole-body composition relative to muscle mass ($P = 0.006$); Vitamin D deficiency in 48%, despite vitamin D supplementation; In IBD cumulative weight-adjusted prednisolone dose > 150 mg/kg for the preceding 3 yr increased the risk for low whole-body areal BMD (OR = 5.5, 95 %CI: 1.3-23.3, $P = 0.02$). Vertebral fractures found in 11% of patients and in 3% of controls ($P = 0.02$)	IBD in childhood was associated with low areal BMD and reduced bone mass accrual relative to muscle mass; The risk for subclinical vertebral fractures may be increased; Careful follow-up and active preventive measures are needed
Ahmed <i>et al</i> ^[60] <i>J Pediatr Gastroenterol Nutr</i> 2004	Prospective cohort study	47 CD and 26 UC (median age of 13.5 yr - range, 5.5-18.2 yr)	Pts with CD were shorter than those with UC ($P < 0.05$); Median ppBone Area for LS and total body for the whole group was 85% and 81%, respectively; ppBone Area at both sites was directly related to height SDS and BMI SDS ($r > 0.5$; $P < 0.005$); Median BMD SDS for LS and total body was -1.6 and -0.9, respectively; Median ppBMC for LS and total bone was 98% and 101%, respectively; ppBMC showed no relationship to ppBone Area ($r = 0.1$, NS); Children with osteopenia 22% after adjustment for bone area	Children with IBD often have small bones for age because they have growth retardation; When DXA data are interpreted with adjustment for bone size, most children have adequate bone mass; Correct interpretation of DXA is important for identifying children who may be at a real risk of osteoporosis
Burnham <i>et al</i> ^[61] <i>J Bone Miner Res</i> 2004	Prospective cohort study	104 children and young adults with CD 233 healthy controls (age 4-26)	CD pts had significantly lower Ht Z-score, BMI Z-score, and lean mass relative to Ht compared with controls (all $P < 0.0001$); After adjustment for group differences in age, Ht, and race, the ratio of BMC in CD relative to controls was significantly reduced in M (0.86; 95%CI: 0.83-0.94) and F (0.91; 95%CI: 0.85-0.98) with CD; Adjustment for pubertal maturation did not alter the estimate; addition of lean mass to the model eliminated the bone deficit; Steroid exposure was associated with short stature but not bone deficits	Importance of considering differences in body size and composition when interpreting DXA data in children with chronic inflammatory conditions; Association between deficits in muscle mass and bone in pediatric CD
Boot <i>et al</i> ^[62] <i>Gut</i> 1998	Prospective cohort study	55 pts (34 M 21 F, age range 4-18 yr) 22 CD, 33 UC	Mean SDS of LS BMD and total body BMD were significantly lower than normal (-0.75 and -0.95, both $P < 0.001$); Height SDS and BMI SDS were decreased. The decrease in BMD SDS could not be explained by delay in bone maturation; The cumulative dose of prednisolone correlated negatively with LS BMD SDS ($r = -0.32$, $P < 0.02$); BMI SDS correlated positively with total body BMD SDS ($r = 0.36$, $P < 0.02$); CD pts had significantly lower LS and total BMD SDS than UC pts, even after adjustment for cumulative dose of prednisolone; In the longitudinal data cumulative dose of prednisolone between the measurements correlated negatively with the change in LS and total BMD SDS; Lean tissue mass measured by dual X-ray absorptiometry had a strong correlation with lean body mass measured by bioelectrical impedance analysis ($r = 0.98$)	IBD children have a decreased BMD; CD children have a higher risk of developing osteopenia than UC children; Corticosteroid therapy and nutritional status are important determinants of BMD in IBD pts

Dg: Diagnosis; pt/pts: Patient/patients; CD: Crohn's disease; UC: Ulcerative colitis; IBD-U: Unclassified IBD; rhGH: Recombinant growth factor; ESR: Erythrocyte sedimentation rate; BMC: Body cell mass; BMI: Body mass index; Ht: Height; Wt: Weight; BMD: Bone mineral density; DXA: Dual-energy X-ray absorptiometry; EEN: Exclusive enteral nutrition; GH: Growth hormone; (P)CDAI: (Pediatric) Crohn's disease activity index; PUCAI: Pediatric ulcerative colitis activity index; M: Male; F: Female.

children have the potential to improve their BMD by the time they reach early adulthood. Children with UC and CD had significantly lower mean BMD Z-scores for the lumbar spine (LS), both at the baseline and after 2 years.

Sub-analyses of the different age groups at the baseline found the lowest BMD values for patients aged 17 to 19 years, be they boys or girls; at follow-up, these patients' BMD had significantly improved, however^[51].

A recent study by Malik *et al*^[54] (Table 2) assessed the frequency of short stature and poor growth, and how they correlated with the course of the disease and the therapy administered in children with CD. The anthropometric and treatment details regarding 116 children showed that mean height SDS was negatively associated with the use of prednisolone ($P = 0.0001$), azathioprine ($P = 0.0001$), or methotrexate ($P = 0.0001$) and with weight SDS (Wt SDS) ($P = 0.0001$)^[54]. Another study by Malik *et al*^[55] (Table 2) focused on growth and disease activity over 12 mo in 36 children with CD who started taking adalimumab. Disease remission was achieved in 78% of these cases, and an overall 42% of the children caught up in terms of their growth. This was more likely to happen for those in remission, taking immunosuppressants, and those starting adalimumab therapy due to an allergic reaction to infliximab. An increase in growth rate was also seen in 15 children who were on prednisolone therapy when they started taking adalimumab. The authors concluded that clinical response to adalimumab therapy is associated with an improvement in linear growth in some children with CD, and that this is more likely for patients entering remission and on immunosuppression, although the effect is not due to a steroid-sparing effect alone^[55].

Another recent cross-sectional cohort study by Laakso *et al*^[56] (Table 2) compared the skeletal characteristics of 80 children and adolescents suffering from IBD with 80 healthy controls matched for age and gender. The IBD patients had a lower bone age (BA)-adjusted lumbar spine, total body bone area BMD ($P < 0.001$ for both), and whole-body bone mineral content (BMC) than controls, after adjusting for height ($P = 0.02$). Lean mass and fat mass z-scores did not differ between the groups, but IBD patients had a lower whole-body BMC relative to muscle mass ($P = 0.006$). Despite 48% of the IBD patients receiving vitamin D supplementation, deficiencies of this vitamin were common. In the IBD group, a cumulative weight-adjusted prednisolone dose > 150 mg/kg for the preceding 3 years increased the risk of low whole-body aBMD ($P = 0.02$). Vertebral fractures (VFs) were found in 11% of patients and 3% of controls ($P = 0.02$). The authors concluded that IBD in childhood is associated with a low aBMD and reduced bone mass accrual relative to muscle mass; the risk of subclinical VFs may increase. These observations warrant careful follow-up and active preventive measures^[56].

There is a well-established relationship between the long-term use of glucocorticoids for any disease indication and a higher risk of osteoporosis and fractures^[57-59], but the relationship between CD or UC and bone loss remains controversial. Inability to achieve peak bone mass when the disease starts in childhood, malnutrition, immobilization, low BMI, smoking, and hypogonadism may all have a part to play in the pathogenesis of bone loss. Although evidence is sparse on the topic of bone health in children and adolescents with IBD, most authors recommend bone health screening, monitoring growth parameters and pubertal development, checking vitamin

D status and vitamin D and calcium intake, and prescribing exercise and nutritional support^[30]. Bone health status should be assessed systematically in patients treated for more than 6 mo, particularly during puberty^[50].

Assessing BMD with dual energy X-ray absorptiometry (DXA) generally involves a comparison with age- and gender-matched reference ranges, and such studies show a high prevalence of osteopenia in children with IBD^[60]. A recent study by Ahmed *et al*^[60] (Table 2) aimed to compare the prevalence of osteopenia using two interpretation methods, one adjusted for age and gender, the other adjusted for bone size and gender. Forty-seven patients with CD and 26 with UC were considered, and the former were found shorter than the latter (median height, SDS, -0.9 vs 0 , $P < 0.05$). The authors concluded that children with IBD often have small bones for their age because they have a growth deficiency. When DXA data were interpreted after adjusting for bone size, most of the children were found to have an adequate bone mass. It is therefore important to interpret DXA findings correctly to identify children who may be at real risk of osteoporosis^[60].

A study by Burnham *et al*^[61] (Table 2) was designed to assess BMC relative to growth, body composition, and maturation in CD cases compared with controls. Whole-body BMC and lean mass were assessed by DXA in 104 CD subjects and 233 healthy controls. CD was associated with significant deficits in BMC and lean mass, relative to height. Individuals with CD had significantly lower z-scores for height and BMI, and a lower lean mass relative to height than controls ($P < 0.0001$). After adjusting for group differences in age, height, and race, males and females with CD had a significantly lower BMC than controls. Steroid exposure was associated with short stature but not with bone deficits. This study pointed to the importance of considering differences in body size and composition when interpreting DXA data in children with chronic inflammatory conditions, as well as showing an association between deficits in muscle mass and bone in pediatric CD^[61].

A study by Boot *et al*^[62] (Table 2) assessed BMD, nutritional status, and determinants of BMD in 55 children with IBD (34 boys and 21 girls, age range 4-18 years; 22 years with CD, 33 years with UC). The mean SDS for lumbar spine BMD and total body BMD were significantly lower than normal (both $P < 0.001$). The SDS for height and BMI were low as well. The decrease in BMD SDS could not be explained by any delay in bone maturation. The cumulative dose of prednisolone correlated negatively with lumbar spine BMD SDS ($P < 0.02$). Patients with CD had significantly lower lumbar spine and total body BMD SDS than patients with UC, even after adjusting for the cumulative dose of prednisolone. The authors concluded that: children with IBD have a reduced BMD; children with CD are at higher risk of osteopenia than children with UC; and corticosteroid therapy and nutritional status are important determinants of BMD in these patients^[62].

A very interesting report from Whitten *et al*^[63] sup-

ported the role of enteral nutrition in improving bone metabolism. The authors enrolled 23 children with newly-diagnosed CD and 20 controls. Children with CD were treated for 8 weeks with EEN, and inflammatory markers, nutritional markers (height, weight), bone markers [C-terminal telopeptides of Type-1 collagen (CTX), and bone-specific alkaline phosphatase (BAP)] were measured before and after the treatment. At diagnosis, children with CD had higher serum CTX than controls ($P = 0.0003$). After the period of EEN, their CTX levels fell significantly ($P = 0.002$), and their serum BAP levels ($P = 0.07$) increased significantly ($P = 0.02$), both normalizing to control levels. This evidence indicates that, as well as reducing inflammation, decreasing disease activity, and improving nutrition in children with newly-diagnosed CD, EEN therapy also normalizes serum markers of bone turnover, suggesting an improvement in bone health^[63].

To sum up, although current therapy for CD is associated with a better growth rate for the first few years, a substantial proportion of children with CD remain short. Depending on the population considered, the prevalence of osteoporosis has been variably reported to range from 12% to 42% in patients with IBD^[13]. While prospective studies suggest sustained bone loss at both trabecular and cortical sites in long-term glucocorticoid users with IBD^[57], a decrease in bone mass is also seen in patients with active CD not using glucocorticoids^[49,50]. Be that as it may, it is strongly recommended that excessively long periods of corticosteroid therapy be scrupulously avoided, particularly for patients of developmental age, and enteral nutrition should be used (whenever possible) as an alternative front-line therapy because it helps to contain the need for corticosteroids and thus limits their unwanted effects on growth, as well as cosmetic issues (which are very important in adolescence)^[22]. Data on vertebral fractures are scarce and there is no agreement about the risk of non-vertebral fractures in patients with CD, though it has been suggested that patients with IBD may carry a 60% higher risk of non-vertebral fractures. The main question is whether all patients with CD should be treated with bone-protecting agents on the assumption that they could all potentially develop osteoporosis, or whether these agents should be used only in patients clearly at risk of osteoporosis and fractures (providing such patients can be identified)^[49].

PUBERTY-RELATED ISSUES IN PEDIATRIC CROHN'S DISEASE

Many nutritional, inflammatory, immunological, and endocrine factors affecting patients suffering from IBD and influencing their growth also have an important impact on the initiation and progression of puberty. The onset of IBD before puberty is frequently associated with an underdeveloped stature and weight, and with patients having a significantly slower growth rate and lower final height by comparison with the parental target. This is more evident in children with CD than in cases of

UC^[64,65]. Other correlations include delayed puberty and menarche, an extended duration of the pubertal phase, and secondary amenorrhea^[64]. Potential causes of late puberty in patients developing IBD in pre-pubertal and pubertal age include^[64]: (1) malnutrition: this correlates mainly with a delay in menarche and sexual maturity. A link has been suggested between late puberty and reduced fat mass, which is normally rich in the aromatases that induce the conversion of androgens into estrogens and the consequent active production of female hormones; and (2) interactions between proinflammatory cytokines and the endocrine system: endocrine functions seem to be disrupted in IBD patients, also due to a direct effect of proinflammatory cytokines, such as TNF- α , IL-6, and IL-1 β , on hormonal feedback mechanisms.

A recent retrospective study by Mason *et al*^[66] (Table 3) aimed to ascertain the impact of CD and UC on the pubertal growth spurt. Pubertal growth was assessed by calculating peak height velocity (HV) SDS (PHV SDS), height SDS at diagnosis, height SDS at PHV, and age at PHV in patients with CD (30 boys, 11 girls) and UC (14 boys and 12 girls). Systemic markers of disease activity were also recorded. Altered pubertal growth parameters were apparent in the CD cases by comparison with the normal population, particularly in boys. In the group as a whole, age (PHV) showed an association with erythrocyte sedimentation rate ($r = 0.4$; $P = 0.005$) and an inverse association with BMI ($r = 0.4$; $P = 0.001$)^[66].

MANAGEMENT OF GROWTH AND PUBERTAL ISSUES IN PEDIATRIC CROHN'S DISEASE

Healthy children grow at an annual rate of 4–6 cm up until puberty, when their rate of growth doubles for over a year^[22]. A declining trend in growth chart percentiles for height and weight arouses the suspicion of growth deficiency vis-à-vis a child's targets for gender and age^[22]. An early diagnosis of CD is fundamentally important, but the early signs of IBD vary and can easily go unnoticed, meaning that a statural growth deficiency and concomitant late puberty quite often precede the intestinal manifestations of the disease^[22]. It is essential to monitor patients' growth, taking their initial height (as measured before the onset of IBD) for reference and routinely reassessing patients as their disease evolves in order to fully appreciate its impact on their growth^[22]. Monitoring patients' growth rate is also important to see how they are responding to therapy over time^[22]. Precise serial check-ups should always include an assessment of patients' pubertal development, which should be correlated with their statural growth. If any discrepancies come to light, action can be taken without delay: radiology is used to establish patients' skeletal age and thus identify their residual potential for growth^[22]. On average, it takes about 12 mo to see any response to treatment in terms of linear growth or pubertal development, so the intervals

Table 3 Summary of the main studies that were reviewed on management of growth and pubertal issues in pediatric Crohn's disease

Ref.	Type of study	Patients	Results	Conclusion
Mason <i>et al</i> ^[66] <i>Horm Res Paediatr</i> 2011	Retrospective cohort study	IBD adolescents 41 with CD, 30 M 11 F 26 with UC, 14 M 12 F	Altered parameters of pubertal growth observed in the CD groups compared to the normal population: In the CD M group, median Ht at Dg was -0.56 ($P = 0.001$) and median age at peak Ht velocity was 14.45 yr ($P = 0.004$) In the CD F group, median Ht at Dg was -1.14 ($P = 0.007$) and Ht at peak Ht velocity was -0.79 ($P = 0.039$). Individually, 8/30 CD M cases had one or more parameter affected: In the whole group, age at peak Ht velocity showed an association with ESR ($r = 0.4$; $P = 0.005$) and an inverse association with BMI ($r = 0.4$; $P = 0.001$)	Disorders of pubertal growth are more likely to occur in CD (particularly M)
Tietjen <i>et al</i> ^[69] <i>Turk J Gastroenterol</i> 2009	Prospective cohort study	40 pts with CD 26 M, 14 F mean age 16.7 yr (median: 17 yr, range: 4-29 yr)	Urinary GH levels were found as normal in CD; Corticosteroid therapy did not appear to be the most responsible factor for growth failure in CD	Growth failure in patients with CD is not caused by GH deficiency; A high PCDAI score has an important impact on impaired growth in children and adolescents with CD
Wong <i>et al</i> ^[70] <i>J Pediatr Endocrinol Metab</i> 2007	Retrospective data analysis	7 pts with CD 5 M	Median chronological age and median difference between chronological age and bone age was 15.9 yr (range, 13.0-17.9 yr) and 1.7 yr (-0.7-3.3 yr), respectively; Median dose of rhGH at T+0 was 0.23 mg/wk (0.15-0.31); Pubertal status remained unchanged in 6/7 patients; Median albumin and C-reactive protein were similar at T+0 and T+6; Median height SDS at T+0, T+6 and T+12 was -2.2 (-4.0 to -1.5), -1.9 (-4.1 to -0.8), -1.9 (-4.1 to -0.7), respectively (NS). Median Ht velocity SDS at T+0 and T+6 was -2.5 (-4.8-1.4) and -0.9 (-5.3 to 3.4), respectively (NS); Positive correlation between percentage change in Ht velocity SDS at T+6 and dose of rhGH at T+0 ($r = 0.8$, $P = 0.03$)	Introduction of rhGH therapy was associated with a cessation in the deterioration in linear growth; An improvement in Ht SDS was not observed over the period of the study
Wong <i>et al</i> ^[71] <i>Clin Endocrinol (Oxf)</i> 2011	Randomized controlled trial in 2 tertiary Children's Hospitals	22 children with IBD 21 with CD	Median Ht velocity increased from 4.5 (range, 0.6-8.9) at baseline to 10.8 (6.1-15) cm/year at 6 mo ($P = 0.003$) in the rhGH group, whereas in the Ctrl group, it was 3.8 (1.4-6.7) and 3.5 cm/yr (2-9.6), respectively ($P = 0.58$); Median percentage increase in Ht velocity after 6 mo in the rhGH group was 140% (16.7%-916.7%) compared with 17.4% (-42.1%-97.7%) in the Ctrl group ($P < 0.001$). No significant differences in disease activity and proinflammatory cytokines at baseline and 6 mo in both groups	rhGH can improve short-term linear growth in children with CD; The clinical efficacy of this therapy needs to be further studied in longer-term studies of growth, glucose homeostasis, and disease status

Dg: Diagnosis; pt/pts: Patient/patients; CD: Crohn's disease; UC: Ulcerative Colitis; IBD-U: Unclassified IBD; rhGH: Recombinant growth factor; ESR: Erythrocyte sedimentation rate; BMC: Body cell mass; BMI: Body mass index; Ht: Height; Wt: Weight; BMD: Bone mineral density; DXA: Dual-energy X-ray absorptiometry; EEN: Exclusive enteral nutrition; GH: Growth hormone; (P)CDAI: (Pediatric) Crohn's disease activity index; PUCAI: Pediatric ulcerative colitis activity index; M: Male; F: Female; NS: Not significant.

between follow-up assessments should never be less than six months^[22].

To prevent and manage growth deficiencies in pediatric IBD patients, we must first establish the most appropriate nutritional, pharmacological, and surgical treatment for their underlying disease: managing their chronic inflammatory status and providing adequate nutrition are two synergically interacting aspects of the same approach^[22]. Ensuring long-term control of active inflammation and administering an adequate intake of nutrients are both fundamental to promoting normal puberty^[64]. Controlled clinical trials have documented a significant correlation between enteral nutrition, a reduced mucosal production of cytokines, and endoscopic healing. Enteral nutrition is potentially capable of inducing remission and

achieving a nutritional recovery. Trials have also established that the effect of exclusively enteral nutrition on the inflammatory picture is influenced by factors such as the disease being localized in the small intestine or of recent onset, whereas the age factor appears to be less influential^[22]. Immunomodulators other than corticosteroids used for pediatric IBD include the thiopurines (azathioprine and 6-mercaptopurine), which are used to maintain remission and have no demonstrated side-effects on growth, and biologics (infliximab and adalimumab), which potentially improve growth velocity by inducing and maintaining disease remission. Artificially-inducing puberty with the aid of estrogens and testosterone carries the risk of causing early growth cartilage calcification, giving rise to statural deficiencies^[64].

Role of treatment with recombinant growth factor for pediatric Crohn's disease

Current treatment strategies for CD that include therapy against TNF- α have been found to improve growth velocity, but linear growth deficiencies persist even with optimized therapy^[67]. Through complex mechanisms that include reducing IGF-1 levels and inducing systemic and hepatic GH resistance, cytokines such as TNF- α and IL-6 - which are commonly elevated in active CD - are important mediators of linear growth delay^[68]. The potential for linear growth impairment as a complication of chronic intestinal inflammation is unique to pediatric CD patient populations^[67]. IGF-I, produced by the liver in response to GH stimulation, is the key mediator of GH effects on the growth plate of bones. There is a well-known association between impaired growth in children with CD and low IGF-I levels. Early studies emphasized the role of malnutrition in suppressing IGF-I production. The direct, growth-inhibiting effects of pro-inflammatory cytokines have been increasingly recognized and explored. The role of non-cytokine factors (such as lipopolysaccharides) and their potential for negatively influencing the growth axis have also been investigated^[67]. Recent evidence suggests that recombinant growth factor (rhGH) therapy is effective in improving short-term linear growth in selected patients^[2], but is of limited benefit as a therapy for improving mucosal disease or reducing clinical disease activity^[67]. A clinical analysis was performed by Tietjen *et al*^[69] (Table 3) on 40 children, adolescents, and young adults with CD to see whether their growth failure was caused by impaired GH secretion. To assess growth hormone excretion, the authors measured urinary growth hormone with an in vitro immunoradiometric assay in three morning urine samples. They found normal urinary growth hormone levels in CD, concluding that growth failure in patients with CD is not caused by GH deficiency. Corticosteroid therapy did not appear to be the main culprit responsible for growth failure in CD either^[69]. A retrospective data analysis was conducted by Wong *et al*^[70] (Table 3) on 7 patients with CD treated with rhGH, after which the deterioration in their linear growth came to a stop, but no improvement in their height SDS was observed during the study period^[70]. Another randomized controlled trial at two tertiary children's hospitals on 22 children with IBD (21 being cases of CD)^[71] (Table 3) investigated the effects of rhGH on HV and glucose homeostasis over a 6-mo period. The median HV increased from 4.5 (range 0.6-8.9) at the baseline to 10.8 (6.1-15) cm/year at 6 mo ($P = 0.003$) in the rhGH group, while in the control group it was 3.8 (1.4-6.7) and 3.5 cm/year (2.0-9.6), respectively ($P = 0.58$). The median percentage increase in HV over 6 mo was 140% (16.7-916.7) in the rhGH group and 17.4% (42.1-97.7) in the control group ($P < 0.001$). There were no significant differences in disease activity or pro-inflammatory cytokines at the baseline or after 6 mo in either group, and the change in bone age for chronological age was also similar in the two groups^[71]. This was

the first randomized controlled trial on rhGH in children with IBD and growth retardation, and it showed - albeit over a brief period of 6 mo - that a dose of 0.067 mg/kg per day of rhGH improves linear growth. The authors also emphasized the continuing need to optimize the child's disease status (*i.e.*, to induce and maintain remission of IBD activity), as they found a greater growth response to rhGH in patients in biochemical remission. In short, although these data provide evidence of the efficacy of rhGH treatment in terms of height velocity over a short- to medium-term follow-up, patients treated with GH experienced no significant improvements in disease activity and pro-inflammatory cytokines by comparison with controls; and long-term follow-up data are lacking. In conclusion, based on currently-available evidence, the efficacy of rhGH in treating growth failure associated with CD is still unclear, and future studies should explore the use of higher doses of rhGH in CD^[70].

CONCLUSION

Despite current treatment strategies for CD including anti-TNF- α medication, short stature and slow growth are still encountered in children with CD. Several studies have shown that children with IBD may not achieve optimal bone mass^[25], and those with CD have multiple risk factors for impaired bone accrual^[22]. A declining trend in growth chart percentiles for height and weight with respect to a patient's targets for gender and age should arouse the suspicion of a growth deficiency^[22]. An early diagnosis is fundamentally important, but signs of the onset of IBD vary and can easily go unnoticed, meaning that statural growth deficiencies and concomitant late puberty quite often precede the intestinal manifestations of the disease^[64].

Nutritional concerns are common in pediatric CD patients, who are often underweight at presentation^[1]. Undernutrition has been reported in up to 65%-75% of such patients^[25], and a low dietary intake due to poor appetite and aversion to food is a major cause of undernutrition in pediatric IBD, though the systemic release of proinflammatory cytokines also contributes significantly^[26]. Although medical treatment can quickly restore body weight, this does not reflect concomitant changes in patients' body composition, which is characterized by normal fat stores but depleted lean mass. Poor bone health, delayed puberty, and growth failure may also complicate these patients' clinical management^[26]. Vitamin and mineral deficiencies have been described in patients with IBD and are attributed to mucosal inflammation in the gut and a low oral intake. Although 25(OH) vitamin D levels have yet to be convincingly demonstrated to correlate with BMD^[56], poor vitamin D status may have detrimental consequences for any child's future health, so an optimal vitamin D status still represents a crucial public health goal^[37]. Corticosteroid therapy and nutritional status are important determinants of BMD in CD patients^[49].

It is indispensable to monitor CD patients' growth, taking their initial height as a reference and routinely reassessing them as their disease evolves in order to fully appreciate its impact on their growth^[22]. Monitoring patients' growth rate is also essential to enable their response to therapy to be assessed over time^[22]. Precise serial check-ups should always include an assessment of patients' pubertal development, which should be correlated with their statural growth, so that action can be taken without delay in the event of any discrepancies coming to light. A radiological examination of patients' skeletal age enables their residual potential for growth to be identified^[22]. Recent evidence suggests that rhGH therapy is effective in improving short-term linear growth for a selected group of CD patients, but is of limited benefit as a therapy for improving mucosal disease and reducing its clinical activity^[70]. Exclusive enteral nutrition is a potentially effective option for treating CD because it can improve patients' nutritional state as well as inducing disease remission (mucosal healing) just as quickly as corticosteroids^[1,27].

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Biological therapy for ulcerative colitis: An update

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blocker (vedolizumab) reportedly achieved good clinical response and remission rates in ulcerative colitis patients. Thus, we believe that the detailed investigation of various studies on clinical trials performed thus far may provide important information for the selection of appropriate biological agents, and therefore, we have extensively reviewed such trials in the present study.

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Abstract

Of the diverse biological agents used for patients with ulcerative colitis, the anti-tumor necrosis factor- α agents infliximab and adalimumab have been used in large-scale clinical trials and are currently widely used in the treatment of inflammatory bowel disease patients. Recent studies have indicated that golimumab, oral tofacitinib and vedolizumab reportedly achieved good clinical response and remission rates in ulcerative colitis patients. Thus, we believe that the detailed investigation of various studies on clinical trials may provide important information for the selection of appropriate biological agents, and therefore, we have extensively reviewed such trials in the present study.

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Key words: Ulcerative colitis; Immune dysfunction; Biological therapy; Remission; Clinical trial; Inflammatory bowel disease

Core tip: In the last two years, the use of the Janus kinase 3 inhibitor (oral tofacitinib) and the $\alpha 4\beta 7$ integrin

INTRODUCTION

Inflammatory bowel disease (IBD), which is broadly classified as ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic intestinal inflammation. Although its causes have not been clearly understood, it is believed to be influenced by genetic susceptibility, changes in the commensal enteric flora, and immune imbalance^[1-5]. Increase in the production of T cells, cytokines, and chemokines, as well as increased trafficking of immune cells is associated with immune imbalance, and is considered as therapeutic targets for the biological treatment of IBD^[6-9]. The current treatment goals of UC include induction of clinical remission, maintenance of clinical remission, and prevention of UC-related complications^[10,11].

Although the therapeutic agents of UC, such as aminosalicylates, corticosteroids, thiopurines, and cyclosporine, are effective in most cases, biological agents are needed in cases where the disease is refractory or intolerant to therapeutic agents. Anti-tumor necrosis factor (TNF)- α agents are biological agents that are relatively safe and have been utilized for a long duration in UC patients. Anti-TNF- α agents appear to exert their effects

Table 1 Summary of ACT1, ACT2, ULTRA1 and ULTRA2 trials for moderate-to-severe ulcerative colitis

Trial	Clinical scenario	Drug	Dosage	Patients (n)	Follow-up (wk)	Outcome and P value
ACT1 Rutgeerts <i>et al</i> ^[12]	Moderate-to-severe active UC	Infliximab	5 mg/kg IV 10 mg/kg IV (intravenously at weeks 0, 2, and 6 and then every eight weeks)	121 122	54	Clinical response at week 8 (placebo/5 mg/10 mg) -37.2%/69.4%/61.5% ($P < 0.001$, $P < 0.001$) Clinical remission at week 8 -14.9%/38.8%/32.0% ($P < 0.001$, $P = 0.002$) Clinical remission at week 54 -8.9%/25.7%/16.4% ($P = 0.006$, $P = 0.149$)
ACT2 Rutgeerts <i>et al</i> ^[12]	Moderate-to-severe active UC	Infliximab	5 mg/kg IV 10 mg/kg IV	121 120	30	Clinical response at week 8 (placebo/5 mg/10 mg) -29.3%/64.5%/69.2% ($P < 0.001$, $P < 0.001$) Clinical remission at week 8 -5.7%/33.9%/27.5% ($P < 0.001$, $P < 0.001$) Clinical remission at week 30 -3.3%/18.3%/27.3% ($P = 0.010$, $P < 0.001$)
ULTRA1 Reinisch <i>et al</i> ^[15]	Moderate-to-severe active UC	Adalimumab	160/80 mg SC (160 mg at week 0, 80 mg at week 2, 40 mg at weeks 4 and 6) 80/40 mg SC (80 mg at week 0, 40 mg at weeks 2, 4 and 6)	130 130	8	Clinical remission at week 8 (placebo/ADA160/80mg/ADA80/40 mg) -9.2%/18.5%/10.0% ($P = 0.031$, $P = 0.833$)
ULTRA2 Sandborn <i>et al</i> ^[16]	Moderate-to-severe active UC	Adalimumab	160/80 mg SC (160 mg at week 0, 80 mg at week 2, and then 40 mg every other week)	248	52	Clinical remission at week 8 (placebo/ADA) -9.3%/16.5% ($P = 0.019$) Clinical remission at week 52 -8.5%/17.3% ($P = 0.004$)

UC: Ulcerative colitis; ADA: Adalimumab.

by inhibiting the large amount of TNF- α present in the deeper layers of colonic tissues in UC patients^[7]. In addition to anti-TNF- α agents, many studies have been performed on the use of molecules involved in varied inflammatory pathways as biological agents. However, in the present study, we aimed to focus on the mechanism of action, clinical outcomes, and future prospects of infliximab, adalimumab, and golimumab (anti-TNF- α agents); tofacitinib (a Janus kinase (JAK) 3 inhibitor); and vedolizumab (an $\alpha 4\beta 7$ integrin blocker).

ANTI-TNF- α AGENTS: CLASSIC OR NEW GENERATION

Infliximab and adalimumab, the most commonly used anti-TNF- α agents at present, are administered intravenously and subcutaneously, respectively, and have been found to be effective for the treatment of moderate-to-severe UC in clinical trials. Anti-TNF- α , which was first officially approved by the US Food and Drug Administration (FDA) for the treatment of CD in 1998, was also approved by the FDA for the treatment of UC in 2010.

Both infliximab - chimeric mouse-human recombinant monoclonal antibody (25% murine and 75% human) - and adalimumab - completely human anti-TNF- α IgG1 - exert their effects by binding to free and membrane-bound TNF- α in order to prevent TNF- α from attaching to TNF-receptor type1/receptor type2. In ACT1 and ACT2 studies that reported the

effects of infliximab in UC patients^[12], no difference in the effect was noted between the 5-mg/kg administration group and the 10-mg/kg administration group, although a marked improvement was observed in the clinical response and remission rates after 8 wk ($P < 0.001$, Table 1), along with a significant improvement in mucosal healing ($P < 0.001$). Similar effects in the clinical remission rate were observed after 30 and 54 wk of treatment (Table 1). No differences in adverse effects were noted between the infliximab and placebo groups. Moreover, infliximab is well known as a useful rescue therapy to avoid colectomy^[13,14].

In the first 8-wk multicenter randomized controlled study that utilized adalimumab, defined as ULTRA 1, the subjects were divided into 160/80 mg and 80/40 mg groups, based on the loading dose, and were then compared with the placebo group^[15]. The clinical remission rate at week 8 in the adalimumab 160/80 mg group was 2 times higher than that of the placebo group, whereas this value in the adalimumab 80/40 mg group did not differ from that of the placebo group. Thereafter, a 52-wk randomized controlled study, defined as ULTRA2, was performed, which indicated that the clinical remission rate at week 52 in the adalimumab 160/80 mg group was 2 times higher than that of the placebo group ($P = 0.004$, Table 1)^[16]. Following a subanalysis, it was observed that the anti-TNF- α naïve patient group exhibited approximately 2 times higher clinical remission rates at week 8 and week 52, respectively compared to the placebo group (21.3% *vs* 11.0%, 22.0% *vs* 12.4%) In the recently per-

Table 2 Studies for the use of Golimumab, Tofacitinib and Vedolizumab for the treatment of ulcerative colitis

Trial	Clinical scenario	Drug	Dosage	Patients (n)	Follow-up (wk)	Outcome and P value
PURSUIT-SC Sandborn <i>et al</i> ^[23]	Moderate-to-severe active UC	Golimumab	100/50 mg SC 200/100 mg SC 400/200 mg SC (2 wk apart)	71 331 331	6	Clinical response at week 6 (placebo/GLM 200/100 mg/GLM 400/200mg) - 30.3%/51.0%/54.9% ($P < 0.0001$, $P < 0.0001$) Clinical remission at week 6 - 6.4%/17.8%/17.9% ($P < 0.0001$, $P < 0.0001$)
PURSUIT-Maintenance Sandborn <i>et al</i> ^[24]	Moderate-to-severe active UC	Golimumab	50 mg SC 100 mg SC (every 4 wk)	151 151	54	Clinical response at week 54 (placebo/GLM 50 mg/GLM100 mg) - 31.2%/47.0%/49.7% ($P = 0.010$, $P < 0.001$) Clinical remission at weeks 30 and 54 - 15.6%/23.2%/27.8% ($P = 0.122$, $P = 0.004$)
Sandborn <i>et al</i> ^[32]	Moderate-to-severe active UC	Tofacitinib	0.5 mg, 3 mg, 10 mg, 15 mg oral (twice daily for 8 wk)	31/33/33/49	8	Clinical response at week 8 (placebo/0.5 mg/3 mg/10 mg/15 mg) - 42%/32%/48%/61%/78% ($P = 0.39$, $P = 0.55$, $P = 0.10$, $P < 0.001$) Clinical remission at week 8 -10%/13%/33%/48%/41% ($P = 0.76$, $P = 0.001$, $P < 0.001$, $P < 0.001$) Endoscopic remission at week 8 -2%/10%/18%/30%/27% ($P = 0.14$, $P = 0.001$, $P < 0.001$, $P < 0.001$)
Gemini 1 Feagan <i>et al</i> ^[41]	Moderate-to-severe active UC	Vedolizumab	300 mg IV (at weeks 0, 2 and then every 8 or 4 wk)	Cohort 1 (225) Cohort 2 (521)	52	Clinical response at week 6 (placebo/Vedolizumab) - 25.5%/47.1% ($P < 0.001$) Clinical remission at week 6 - 5.4%/16.9% ($P = 0.001$) Clinical remission at week 52 (placebo/Vedolizumab every 8 wk/Vedolizumab every 4 wk) - 15.9%/41.8%/44.8% ($P < 0.001$, $P < 0.001$)

UC: Ulcerative colitis; GLM: Golimumab.

formed study on the effects of adalimumab on hospitalization for UC, the first 8 wk of adalimumab therapy indicated a significant reduction in the risk of all-cause, UC-related, and UC- or drug- related hospitalization compared to the placebo group (40%, 50%, and 47%, $P < 0.05$ for all comparisons)^[17]; however, significant differences were not observed in the rates of colectomy between the groups. The adalimumab and placebo groups did not show any differences in the adverse events^[15,16,18]. The primary failure rate of anti-TNF induction therapy is reportedly 40% in IBD clinical trials; when switching to another anti-TNF agent, the treatment becomes effective at 50%^[19]. A secondary loss of response can also occur at 1 year after anti-TNF initiation in IBD patients^[19], and solutions for the issues in anti-TNF- α treatment of UC are expected to be elucidated in the future.

Golimumab - a novel, completely human IgG1 anti-TNF- α antagonist - is subcutaneously administered and is approved for use in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis patients^[20-22]. As shown in the PURSUIT-SC study, at week 6, the clinical response and remission rates showed a noticeable change in both the golimumab 200/100 mg and 400/200 mg groups (all $P < 0.0001$, Table 2)^[23]. The PURSUIT-maintenance study, which is a phase 3, placebo-controlled, randomized withdrawal study, compared the clinical response and remission rates between the golimumab 50/100 mg group and placebo group up to week 54 at intervals of 4 wk; they observed that a notable change was observed in the golimumab 100 mg

administration group ($P < 0.001$, $P = 0.004$, Table 2)^[24]. After adjusting for the follow-up duration, no difference was noted in adverse events between the placebo and golimumab 100 mg groups.

PROMISING JAK1/JAK3 INHIBITOR AND INTEGRIN BLOCKING ANTIBODY

The JAK-STAT pathway is associated with inflammation, autoimmune diseases, hematopoietic disorders, and transplant rejection^[25-30]. Tofacitinib (formally known as CP-690550) is a selective oral inhibitor of JAK 1 and 3, which is known to inhibit the differentiation of pathogenic Th1 and Th17 cells and innate immune cell signaling^[31]. The effects of tofacitinib in active UC patients showed clinical response rates at week 8 of 32%, 48%, 61%, and 78% for the 0.5 mg, 3 mg, 10 mg, and 15 mg twice daily groups, respectively. The tofacitinib 3 mg, 10 mg, and 15 mg twice daily groups exhibited marked differences in clinical and endoscopic remission rates compared to the placebo group (all $P \leq 0.001$, Table 2)^[32]. The levels of low-density and high-density lipoprotein cholesterol increased in a dose-dependent manner, and an absolute neutrophil count of < 1500 was observed in 2% of patients in the tofacitinib group. Thus, tofacitinib is considered to be an effective and safe drug for moderate-to-severe UC patients.

$\alpha 4\beta 7$ integrin, a molecule that is expressed on circulating B and T lymphocytes, interacts with the ligand of the mucosal addressin-cell adhesion molecule

(MAdCAM-1)^[33,34]. The bound lymphocyte migrates to the lamina propria and tissues, and then induces the inflammatory cascade^[35]. Vedolizumab - a humanized monoclonal antibody that inhibits the binding of $\alpha 4\beta 7$ integrin complex and MAdCAM-1-selectively blocks gut lymphocyte trafficking^[36,37], and thus demonstrates therapeutic effects in IBD patients^[38,39]. Consequently, unlike natalizumab which is a $\alpha 4\beta 7$ and $\alpha 4\beta 1$ integrin antagonist, vedolizumab does not affect the cerebrospinal fluid T-lymphocyte immunophenotype and therefore, it does not cause progressive multifocal leukoencephalopathy^[40]. When vedolizumab (300 mg) was administered at week 0 and week 2 and then administered at intervals of 4 or 8 wk, a marked response in the clinical response and remission rates was noted after week 6 ($P < 0.001$, $P = 0.001$, respectively, Table 2)^[41]. A noticeable change in the clinical remission rate at week 52 was observed, regardless of whether the medication was administered at 4- or 8-wk intervals (all $P < 0.001$).

CONCLUSION

Biological agents have been used for UC treatment for 10 years, and various types of biological agents have been developed and used worldwide, with the most common being anti-TNF- α agents. This increase in the development of biological agents provides further immunologic information in addition to offering a wide range of drugs for use. Thus, biological agents may serve as another appropriate option for clinicians in the treatment of UC patients who may not be effectively treated with conventional drugs. Since an accurate understanding of biological agents can be achieved through clinical trials, performed as part of large-scale randomized controlled studies, we have reviewed them in detail. In the future, we believe that biological agents with superior therapeutic effects and fewer side effects, compared to those used currently, will be developed, thus bridging the therapeutic gap present in the treatment of UC patients.

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Predictive proteomic biomarkers for inflammatory bowel disease-associated cancer: Where are we now in the era of the next generation proteomics?

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among patients diagnosed with IBD, and the discovery of proteome biomarkers to diagnose or predict cancer risks. Host genetic factors influence the etiology of IBD, as do microbial ecosystems in the human bowel, which are not uniform, but instead represent many different microhabitats that can be influenced by diet and might affect processes essential to bowel metabolism. Further advances in basic research regarding intestinal inflammation may reveal new insights into the role of inflammatory mediators, referred to as the inflammasome, and the macromolecular complex of metabolites formed by intestinal bacteria. Collectively, knowledge of the inflammasome and metagenomics will lead to the development of biomarkers for IBD that target specific pathogenic mechanisms involved in the spontaneous progress of IBD. In this review article, our recent results regarding the discovery of potential proteomic biomarkers using a label-free quantification technique are introduced and on-going projects contributing to either the discrimination of IBD subtypes or to the prediction of cancer risks are accompanied by updated information from IBD biomarker research.

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Key words: Inflammatory bowel disease; Biomarker; Proteomics; Tailored medicine; Colitic cancer

Abstract

Recent advances in genomic medicine have opened up the possibility of tailored medicine that may eventually replace traditional "one-size-fits all" approaches to the treatment of inflammatory bowel disease (IBD). In addition to exploring the interactions between hosts and microbes, referred to as the microbiome, a variety of strategies that can be tailored to an individual in the coming era of personalized medicine in the treatment of IBD are being investigated. These include prompt genomic screening of patients at risk of developing IBD, the utility of molecular discrimination of IBD subtypes

Core tip: Our recent achievements in discovering biomarkers to predict cancer risk are introduced. Ultimately, models based on combinations of genotype and gene expression data referenced with clinical, biochemical, and serological data may permit the development of tools for individualized risk stratification and efficient treatment selection, as well as complete rescue from complications, including colitis-associated cancer, in the near future.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disease that causes injury to the gastrointestinal (GI) tract and is accompanied by clinical characteristics of remission and relapse. The two common types of IBD are ulcerative colitis (UC) and Crohn's disease (CD). Although many molecular methods for the investigation of protein and gene sequences have contributed to diagnostic methodologies, the diagnosis of IBD is primarily based on clinical, endoscopic, radiological, and histological criteria. Unfortunately, there has been little to no change in this traditional approach to diagnosis, despite modern advances in genomics and proteomics. However, progress in treatment strategies involving the incorporation of marketed biologicals and molecular targeted therapeutics has led to the development of the concept of "deep remission" or "mucosal healing" in the treatment of IBD^[1,2]. Furthermore, there has been major innovation in diagnostic methods, including the development of more complicated endoscopic and non-invasive imaging methods. These techniques are used to improve quality of life (QOL) of patients, predict complications, and contribute to the prevention or surveillance of cancer associated with IBD such as colitis-associated cancer (CAC)^[3].

Recent developments in the molecular pathogenesis of IBD have highlighted three aspects. First, IBD is caused by complex disorders influenced by susceptibility genes, and is characterized by disturbed epithelial barrier function, and abnormal innate and adaptive immunity. Second, the compositions of gut microflora are altered or the epithelial barrier function is disorganized, which leads to a response from the immune system. Third, a murine model has been very helpful in unraveling the pathogenesis/mucosal immunopathology of IBD^[4] by suggesting that the abnormal immune reaction to normal microbiota results from dysregulation of the mucosal immune system^[5]. For example, the composition of microbes in the gastrointestinal tract may impair the patient's lifestyle in developed countries and a pathogenic infection in the gastrointestinal tract has a significant function in modulating the immune system. These data may explain why developing and some Asian countries are confronting steep increases in the incidence of IBD. Developments in gene-sequencing technologies, such as next generation sequencing, as well as the emergence of several bioinformatic tools have led to novel insights into the microbe balance in the human gastrointestinal tract and the effect

of microbes on human physiology and pathology^[6].

Additional innovative technologies, such as mass spectrometry (MS)-based proteomics, also referred to as next generation proteomics, have discovered new classes of proteomic biomarkers that can be used to explore the accurate and comprehensive molecular characterization of IBD genes and proteomes. These advances are expected to lead to more reliable identification of IBD diagnostic- or progression-specific targets and enable molecular diagnosis, as well as provide guidance regarding the selection of treatment options and the risk of cancer development in cases with longstanding remission and relapse^[7]. A more robust molecular definition of IBD subtypes is likely to be based on specific molecular pathways that determine not only disease susceptibility, but also disease characteristics, such as location, natural history and therapeutic response. Furthermore, such advances could be applicable in defining "deep remission", which has not been feasible with currently-used scoring systems or endoscopic evaluation. Discovering biomarkers for IBD may allow objective measurements of disease activity and severity while also serving as prognostic indicators for therapeutic outcomes^[8]. Furthermore, the discovery of one or more biomarkers predictive of the risk of IBD-associated cancers, such as CAC, combined with advanced therapeutics, may lead to tremendous improvements in patient QOL in the near future.

In this review article, our recent achievements in discovering biomarkers to predict cancer risk are introduced. Ultimately, models based on combinations of genotype and gene expression data referenced with clinical, biochemical, and serological data may permit the development of tools for individualized risk stratification and efficient treatment selection, as well as complete rescue from complications, including CAC, in the near future^[9].

Molecular pathogenesis of colorectal cancer and CAC

Chronic inflammatory diseases are associated with cancer incidence, which is dependent on the duration and severity of the diseases. For example, Barrett's esophagus is relevant to esophageal cancer, chronic *Helicobacter pylori*-associated chronic atrophic gastritis is relevant to gastric cancer, and UC or CD are relevant to CAC. These are well-acknowledged examples that support a connection between gut inflammation and cancer. In fact, it has been reported that patients with IBD are at enhanced risk of colorectal cancer (CRC): approximately 15% of CRC patients have related IBD etiology^[10]. Although general carcinogenesis is a multi-factorial process that combines accumulation of genetic mutations, post-translational modification, and cell-matrix reciprocal action, inflammation-prone carcinogenesis is somewhat different^[11]. The utility of biomarkers for CAC can be extended to permit earlier detection of dysplasia; therefore, the targeted manipulation of biomarkers might lead to advances in cancer therapies and cancer preventions, and may prove to be effective in reducing the development of CAC, with clinical interventions such as blocking agents or

endoscopic treatments. Regarding molecular aspects, the mechanism of CAC in IBD differs from CRC, which is a well-known adenoma-to-carcinoma sequence. CAC appears to take place from either flat dysplastic tissue or dysplasia-associated lesions or masses^[12]. The major pathways of sporadic CRC and CAC comprise chromosomal instability, hypermethylation and microsatellite instability; however, CAC shows inflamed colonic mucosa before histological changes of dysplasia or cancer. Patients with IBD have a high risk of CAC following diagnosis, and patients have common symptom, such as colitis^[13]. The risk of CAC is increased in younger patients, those with more extensive colitis, those with concomitant primary sclerosing cholangitis, and a family history of CRC^[14]. Most cancers show no high risk related to proctitis; however, increased in pancolitis, *i.e.*, left-sided colitis, carries an intermediate cancer risk^[15]. Patients with CD and UD have the same risk of CRC and the prevalence in the US is greater than 200 cases per 100000, representing a total of between 1 and 1.5 million patients with IBD. Fortunately, the incidence of CAC is lower than CRC in the United States and other western countries^[16]. A biological background for the high risk of CRC in IBD gives a one-sided interpretation, patients with high levels of inflammatory mediators production may progress to CAC. The key signal of IBD-induced carcinogenesis is inflammatory cytokines induced by mucosal and immune cells in the gut. The key molecules of inflammation, including nuclear factor kappaB (NF- κ B) and cyclooxygenase-2 (COX-2), are important links between inflammation and cancer. Recently, other factors, such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) -induced signaling, have been proven to induce cancer development in animal models of CAC^[17]. Based on these aspects of molecular carcinogenesis, we have tried to apply biologicals such as infliximab to neutralize TNF- α , a proton pump inhibitor based on mechanisms including the inhibition of NF- κ B as well as attenuation of oxidative stress, and aspirin/celecoxib to inhibit cancer-prone COX enzymes. As expected, all of these efforts to block inflammation-promoted carcinogenesis efficiently prevented CAC in our mouse model experiments^[18,19]. The recent descriptions of epigenetic alterations, in particular alterations in DNA methylation, that have been observed during inflammation and inflammation-associated carcinogenesis, led us to explore nutritional interventions as a means of targeting and correcting epigenetic oncogenic abnormalities, as a form of CAC prevention^[20].

Prediction of CAC

Patients with long-standing UC and CD are at increased risk of developing CRC, and patients with small intestinal CD have a high risk for developing small bowel adenocarcinoma. Unlike the sporadic CRC that can develop in those with IBD, CAC development is intimately associated with IBD. In those with IBD, CAC results from a process that is believed to begin with mutagenic benign inflammation that develops into indeterminate, low-grade

and high-grade dysplasia, and eventually to carcinoma. Regarding the risk factors predisposing to carcinoma in IBD, the risk is increased depending on duration, severity of colitis, presence of sclerosing cholangitis, degree of inflammation and family history of CRC. Evidence-based medicine advises that patients with colitis should be kept under surveillance colonoscopy after diagnosis for 8 to 10 years. As surveillance guidelines for early detection of CAC, the general approach of periodic endoscopic examinations and systematic random biopsies of involved mucosa is generally recommended^[21]. Recently, advanced colonoscopic techniques, including narrow band imaging, chromoendoscopy and confocal microendoscopy, have been used to identify abnormal areas in targeted, but not random, biopsies, and biomarkers could be adopted for high resolution endoscopy^[22]. Although medications such as aminosalicylates, folic acid and ursodeoxycholic acid seem to be chemopreventive, potent preventive therapeutics, as well as surveillance of high risk patients through the use of potential biomarkers, would seem to be ideal^[23].

Current Status Of Biological Markers For IBD

Serological markers for IBD are rapidly developing. However, the most studied antibodies, anti-*Saccharomyces cerevisiae* antibodies (ASCA) and atypical perinuclear antineutrophil cytoplasmic antibodies (P-ANCA), have limited sensitivity. Thus, the relationship between serological markers of disease pattern and phenotype may be of greater value than the use of serological markers as diagnostic tools. For example, patients with CD who have high titers of various serological makers have more serious small intestine disease than those with low titers of antibodies^[24]. On the genetic level, application of a genome wide association study design in CD has provided new insights into the immunopathogenesis of CD, identifying links to genes of the innate and adaptive immune system^[25]. One patient had CD associated with gene mutations of *NOD2* and the *ATG16L1* autophagy gene, both of which affect the intracellular processing of bacterial components. In addition, genetic variation of the IL-23 receptor, *STAT3* and *NKX2-3* genes, were associated with CD and UC in Asian patients. Although comparative analyses of gene associations between CD and UD can identify unique mechanisms of immunopathogenesis of IBD, such results have limited applicability in real-world clinical settings because of ethnic, racial, and environmental differences in the samples studied. Since the advent of the concept of proteomics, a plethora of proteomic technologies have been developed to study proteomes. In IBD, several studies have used proteomics to better understand the disease and discover molecules that could serve as therapeutic targets. The advance of proteomic technologies will have an important effect on the development of new biomarkers for IBD^[26]. Further advances in proteomic technologies have allowed us to use label-free quantification to detect biomarkers in various IBD patients for the first time. The results are

expected to provide additional insights in to the molecular biomarkers of IBD that may be used in predicting responses to treatment^[27].

Classic serological and fecal markers in IBD

Currently, diagnosis of IBD from the blood and stool of patients represent reliable and quantitative tools to clinicians^[28]. The C-reactive protein (CRP) and fecal-based leukocyte markers, calprotectin (Cal) or lactoferrin (Lf), can help clinicians to assess disease activity and to distinguish IBD from non-inflammatory diarrhea and simple colitis. Serological tests including both ASCA and P-ANCA can be used to determine the current status and risk of IBD^[29]. The progression of IBD and inflammatory processes are assessed by tests for CRP and the erythrocyte sedimentation rate (ESR). In addition, clinicians might measure the levels of drug metabolites and antibodies against therapeutic agents that aim to determine why patients do not respond to treatment and to select alternative therapy. The advantages of using the fecal markers including, Cal or Lf, are their ease of detection and use of an inexpensive ELISA technique, as well as their long-term stability in feces^[30]. However, several limitations have been associated with these classical serological and fecal markers in IBD. The ESR technique is simple to perform, widely available, and inexpensive; however, it has several disadvantages, such as a concentration that depends on age, several confounders, and the use of certain drugs^[31]. Several factors can affect the utility of CRP, including long half-life and prolonged latency period after changes in chronic IBD. Determination of fecal Cal or Lf markers is very helpful into diagnosis chronic IBD, while other GI diseases, ischemic colitis, and non-steroidal anti-inflammatory drug-associated intestinal damage show greater leukocyte elimination in feces^[32]. In spite of 80%-100% diagnostic accuracy levels, fecal markers are not specific for IBD and may be elevated in a range of organic conditions. To compensate for these limitations, Langhorst *et al.*^[33] evaluated fecal levels of PMN-elastase (PMN-e) in addition to the aforementioned markers and concluded the IBD and IBS can be discriminated by the fecal markers Cal, Lf, and PMN-e. However, these fecal markers have shown a similar capacity to indicate endoscopic pathology, and are more efficient for diagnosis than CRP. Therefore, the combined diagnosis using fecal markers and CRP with disease-specific activity index will be very useful when assessing endoscopic inflammation in UC. It should be noted that these fecal markers were proven to be very efficient in diagnosing IBD as well as in predicting impending clinical relapse in pediatric patients with IBD^[34]. Despite the benefits that can be derived from these serum and fecal biomarkers, there remains considerable room for improvement regarding disease prediction and prognosis assessment, as none of them can be applied to predict future risk of CAC development in IBD.

Proteomic biomarkers in IBD

Matrix-assisted laser desorption/ionization time-of-flight

(MALDI-TOF) mass spectrometry (MS) and surface-enhanced laser desorption/ionization (SELDI)-TOF MS, have become popular methods recently for the analysis of macromolecules of biological origin such as tissues, serum, or plasma^[35]. MALDI-TOF MS is used in clinical medicine to identify disease markers in combination with classic protein gel analysis (1-D) and two dimensional gel electrophoresis separation, accomplished through either peptide mass fingerprinting or peptide sequence tag (2-D) analysis followed by a data base search using proteome blot analysis software^[36]. Using these applications, evaluation of samples by MALDI-TOF MS can give novel data regarding peptides present at high molecular mass and may therefore be valuable to assess potential disease markers of IBD. For example, Nanni *et al.*^[37] determined serum proteins of 22 healthy subjects and 41 patients with IBD (15 CD, 26 UC) extracted with reversed-phase (C18) and subsequently performed MALDI-TOF MS. The results of serum protein profiles showed the highest overall prognosis capability (96.9%) of identifiable protein biomarkers involved in IBD discrimination. Similarly, in a study by Liu *et al.*^[38], serum proteins from 74 CRC samples compared with 48 healthy samples were applied to SELDI-TOF MS using a ProteinChip reader. The diagnostic pattern could distinguish samples according to status of CRC from normal samples with sensitivity and specificity of 95% by independent analysis of the samples. These two studies demonstrated the high potential for biomarker discovery in patients with IBD or CRC in clinical settings, and further clinical validation in large patient cohorts is expected to promote the use of novel biomarkers in clinical practice^[39]. In addition to protein identification, 2-D difference gel electrophoresis (2D DIGE) is good method protein quantification; however, isobaric tags for relative and absolute quantification (iTRAQ) and stable isotope labeling by amino acids (SILAC) are the best methods developed recently. Sample preparation is important to the success or failure of such analysis and subcellular fractionation can be used to give more specific protein localization analysis than total cellular proteins^[40-42]. In our recent study^[43], we applied advanced technologies such as iTRAQ and SILAC for label-free quantification in samples obtained from a mouse model of UC and CAC to identify potential biomarkers for cancer-prone inflammation in IBD and to evaluate empirical therapeutics.

PROTEOMIC BIOMARKER DISCOVERY FOR CAC: A CLASSICAL PROTEOMIC APPROACH

Multiple chemical and biological systems, including intestinal tissue, its associated immune system, the gut microbiota, xenobiotics, and their metabolites, meet and interact to form a tightly regulated state of tissue homeostasis. Disturbance to this state of homeostasis can cause IBD as well as CAC through intercalated multi-factorial

mechanisms. Many strong pathological and mechanistic correlates exist between mouse models of CAC and the clinically relevant situation in humans, allowing for the use of systems biology approaches^[44]. Furthermore, the close proximity of colonic tumors to the myriad of intestinal microbes, as well as the instrumental implication of microbiota in IBD, introduces microbes as new factors capable of triggering inflammation and possibly promoting CAC, necessitating high throughput metabolomic approaches^[45]. Additionally, a detailed understanding of these interactions may also provide a means of preventing CAC^[46]. In a small study, Watanabe *et al*^[47] performed a low density array analysis of 149 genes implicated in CAC and identified 20 genes showing differential expression between UC- and non-UC-associated CAC, including cancer-related genes such as *CYP27B1*, runt-related transcription factor 3, sterile alpha motif domain-containing protein 1, EGF-like repeats and discoidin I-like domain 3, nucleolar protein 3, *CXCL9*, integrin beta2, and *LYN*. Colliver *et al*^[48] demonstrated that 392 transcripts showed differential expression during progression from UC to CAC. Both dysplasia and CAC showed 224 transcripts in common and it was concluded that some genes showed same modification both in dysplasia and CAC, signifying that they might be related to tumor initiation and progression. Following these studies, a host of potential biomarkers have been reported in the literature, including cytokeratin 7/20^[49], a-methylacyl-CoA-racemase^[50], transgelin, a frame shift mutation in the TGF- β type II receptor^[51,52], HSP47^[53], methylation of the estrogen receptor^[54], association with certain HLA class II alleles^[55], DNA methyltransferase-1^[56], 8-nitroguanine or 8-oxo-deoxyguanine^[57-59], CCL20^[60] and activation-induced cytidine deaminase^[61]. We used our experimental animal model for colitic cancer, which was provoked with repeated bouts of UC, and an additional proteomic method based on 2-D electrophoresis and MALDI-TOF MS to analyze proteins related in CAC. In detail, 38 proteins were differentially expressed between CAC and healthy samples, using comparative 2-D electrophoresis analysis. Through validation studies, 27 proteins, including enolase, GRP94, HSC70 prohibitin and transgelin, were identified. Among these identified proteins, the downregulation of transgelin in mouse colitic cancer was supported by western blotting and immunohistochemistry. Moreover, transgelin was significantly decreased in colon tumors compared with non-tumorous regions in humans, implying that reduced levels of transgelin could be a good biomarker for CAC^[62].

BIOMARKER DISCOVERY FOR CAC: THE NEXT GENERATION OF PROTEOMICS

Currently, knowledge of proteomics is important and provides researches with complicated label and label-free techniques. This next generation of proteomics may provide effective perspectives in the diagnosis and treatment of gastrointestinal disease and allow for translation of proteomics from the bench to the bedside. Currently,

proteomic studies focus not only in the identification of proteins in a sample, but also the quantification of them. Various protein expression profiles are examined because they provide useful information, especially in clinical proteomics, involving molecular targets related to specific diseases. The technology for proteomics study is continuously developing, and both of labeled and label-free methods have their several advantages. Currently, label-free and isotope-label techniques are used to study proteins quantitatively. The label-free approach, combining spectral and signal quantitation, for identifying amino acids provides accurate relative protein expression data and is an easy way to determine quantitative information. However, this strategy is subject errors from variation in protein preparation that may be reduced when various stable isotopes are inserted in the specimens to make protein isotopomers, which have different spectra according to their different masses. Therefore, several metabolic labeling strategies that apply stable isotopes to minimize error have been developed recently and have been applied in animal models.

Biomarkers to predict CAC risk discovered via label-free quantification analyses

A comparative label-free quantification analysis was conducted in eight patients with UC, eight patients with CD and eight patients with irritable bowel syndrome (IBS). Colonic tissue biopsies were obtained during colonoscopy after written consent and stored in a deep freeze until the assay. Using an Agilent HPLC-Chip 6520 Q-TOF MS system and a label-free quantitative technique (IDEAL-Q v1.0.6.3), signal pathway analysis associated with carcinogenesis was conducted to discover potential biomarkers in CAC (Figure 1). The analysis was conducted according to the degree of intestinal inflammation, type of IBD, and extent of inflammation. To compare against the analysis from IBS samples, the proteins implying CAC risk were isolated (Figure 2A and B). As seen in Figure 2A, 22 significant proteins were found to be potential biomarkers predicting CAC risk in patients with UC. Figure 2B shows the 19 proteins found to be potential biomarkers for CAC risk in patients with CD. Further analysis yielded four important protein biomarkers: proteoglycan 2 (PRG2), S100A6 (calcylin), ribosomal protein L18 (RPL18), UDP-glucose dehydrogenase (UGDH) as potential target proteins and predictive biomarkers for CAC risk in IBD (Figure 3). PRG is a major component of the animal extracellular matrix and has been shown to be involved in the differentiation process across the epithelial-mesenchymal axis. It is a potential biomarker inferred principally through its ability to bind growth factors and modulate their downstream signaling; malignant tumors have their individual characteristic PRG profiles closely associated with their differentiation and biological behavior. PRG2 has further been implicated as a biomarker for neuropathic pain attributable to advanced pancreatic cancer^[63] in an animal model of CAC^[27], and as an inflammation related gene of several cancers, including prostate,

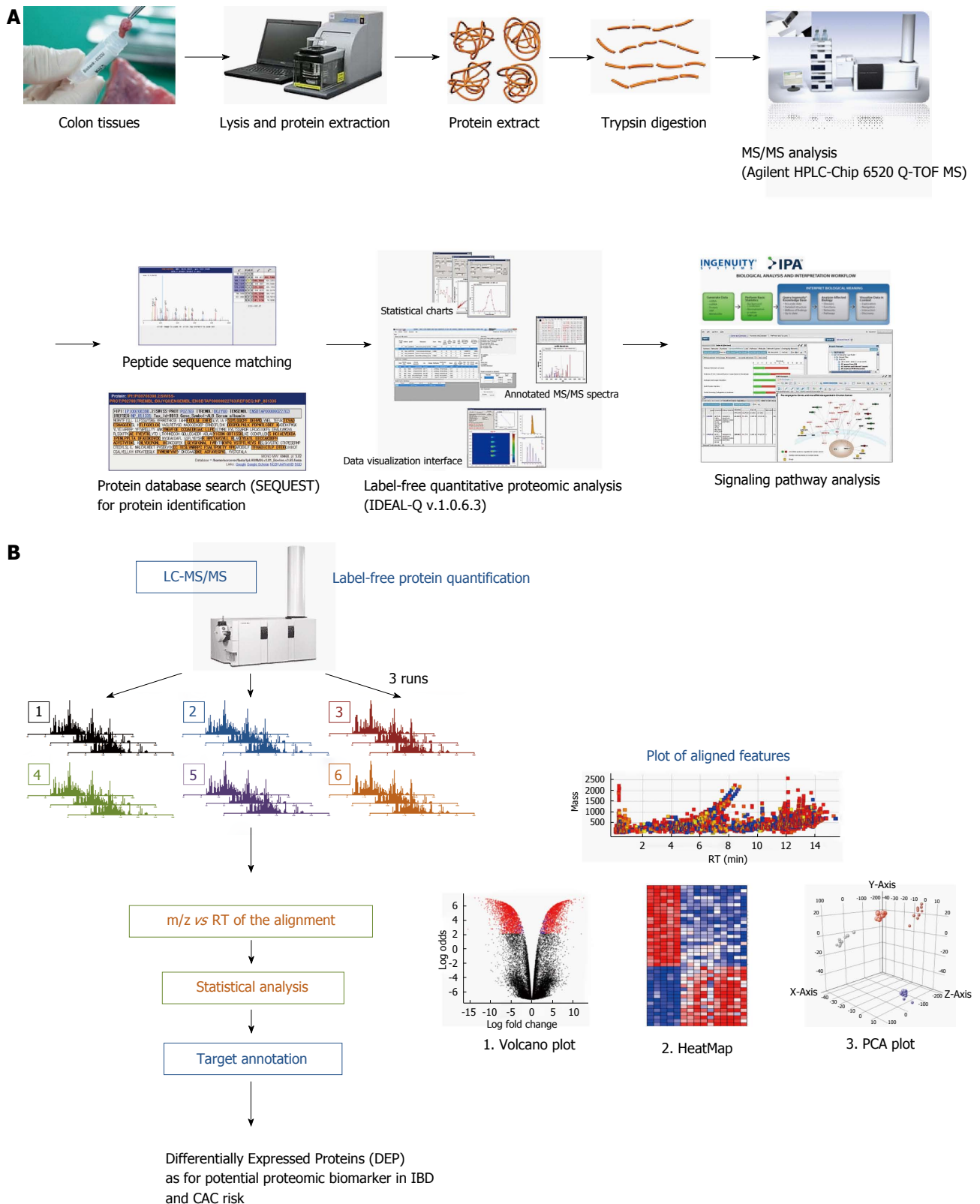


Figure 1 Schematic presentation showing proteome analysis to discover potential biomarkers and label-free quantification analysis in inflammatory bowel disease. A: Applying the label-free quantification method to discover proteomic biomarkers in patients with different types and different stage of inflammatory bowel disease (IBD). Comparative analysis was done in eight patients with ulcerative colitis (UC), eight patients with Crohn's disease (CD) and eight patients with irritable bowel syndrome (IBS). Biopsied colon tissues were obtained during colonoscopy after written consent, and stored in a deep freeze until assayed. Using Agilent HPLC-Chip 6520 Q-time-of-flight mass spectrometry (TOF-MS) and label-free quantitative proteome analysis (IDEAL-Q v1.0.6.3), significant signal pathway analysis was done. In the current review, the analysis done according to the degree of intestinal inflammation, type of IBD, and extent of inflammation from 24 patients, eight from non-IBD normal patients; *i.e.*, IBS patients, eight from patients with UC, and from patients with CD; B: Label-free protein quantification scheme for potential biomarker for colitis-associated cancer (CAC) risk in 16 patients with IBD.

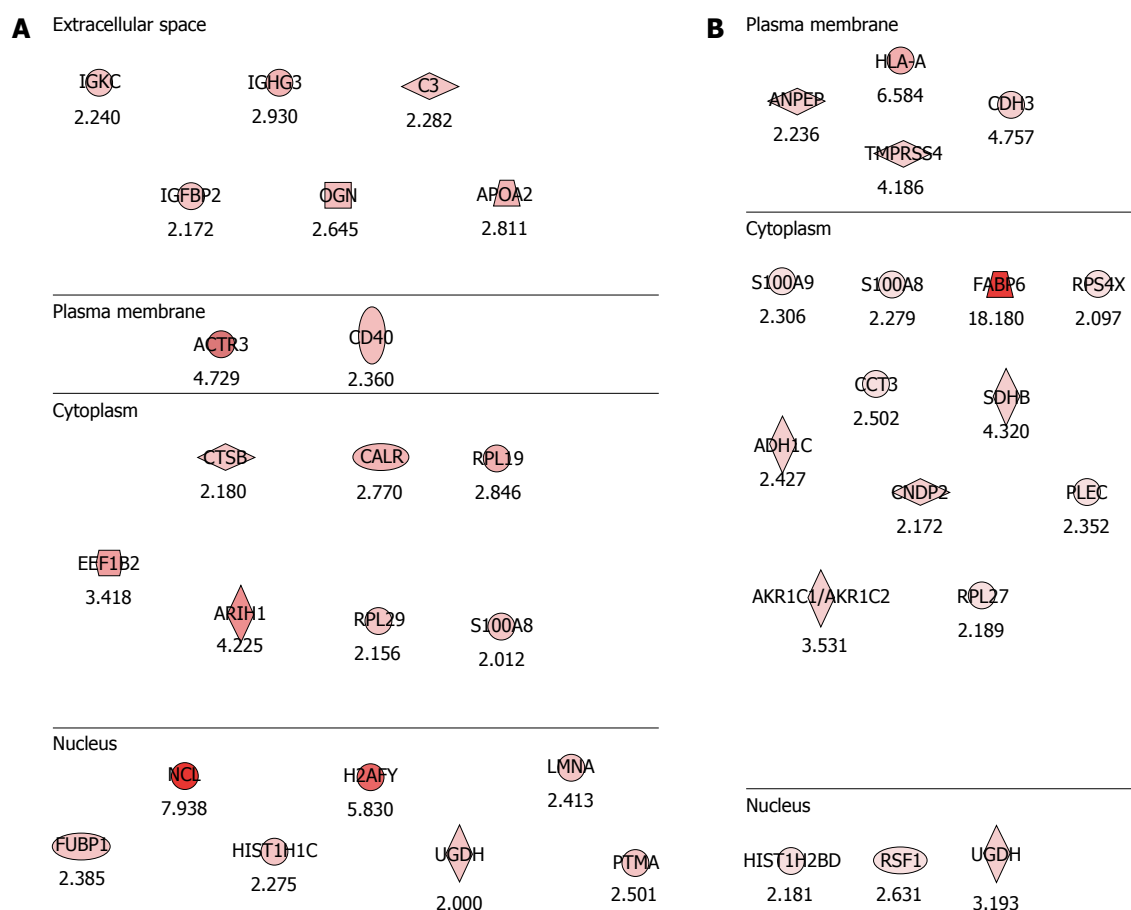


Figure 2 Potential proteomic markers signifying colitis-associated cancer risks in inflammatory bowel disease. A: Proteomic markers for colitis-associated cancer (CAC) risk in patients with ulcerative colitis (UC). The analysis was performed according to the degree of intestinal inflammation, type of inflammatory bowel disease and extent of inflammation. Compared with the analysis of CAC, 22 significant potential biomarkers of CAC risk were obtained from patients as p with UC, six biomarkers existing in the extracellular space, two biomarkers at the plasma membrane, seven are from the cytoplasm, and seven are nuclear proteins; B: Proteomic markers for CAC risk in patients with Crohn's disease (CD). Eighteen potential biomarkers for the risk of CAC were identified in patients with CD. Four were from the plasma membrane, 11 from the cytoplasm, and three from the nucleus.

lung, CRC^[64] and pancreatic cancer^[65]. The expression of S100 calcium binding protein A6 (S100A6) is upregulated in proliferating and differentiating cells^[66], and has been reported to be a possible biomarker for hepatocellular carcinoma^[67,68], pancreatic cancer^[69], acute lymphoblastic leukemia^[70], CRC^[71] and breast cancer^[72]. RPL18 is a 60S ribosomal protein expressed in stem cells^[73] and during adipogenesis^[74]. UGDH has been suggested as biomarker for cancer metabolism. The oxidation of UDP-glucose is catalyzed by UGDH to generate UDP-glucuronic acid (UDP-GlcA), a precursor of glycosaminoglycans (GAGs). Wang *et al.*^[75] showed decreases in the expressions of UGDH, UDP-GlcA and GAG expression after treatment with a UGDH-siRNA in HCT-8 colon cancer cells and concluded that UGDH could be a new target for CRC clinical treatment^[76]. Additionally, UGDH has been identified as a potential biomarker for prostate cancer^[77,78], hepatocellular carcinoma^[79] and breast cancer^[80].

Biomarkers to predict CAC risk discovered via label-based protein quantification analyses

Proteomic techniques with blood and biopsy provide reliable and accurate tools that provide support to clinicians

in the diagnosis and treatment of IBD. For example, clinically meaningful biomarkers may be used in the differential diagnosis of CD and UC, or as predictors of treatment responses. Tandem mass spectrometry (MS/MS) is commonly used proteomic analysis. However, various workflows are possible for peptide analysis before MS/MS, as well as bioinformatics, to identify peptides, for which 2-D electrophoresis and subsequent MS, liquid chromatography-MS, 2D DIGE, and iTRAQ are under development. In our previous publication^[43], the present status and perspectives regarding these developed proteomic methods were discussed, with descriptions of examples of new biomarkers for the diagnosis, treatment and prognosis of IBD and CAC in mouse models and in humans. In detail, we showed new concepts and technologies of proteomics, such as protein identification and proteome coverage, as determined by iTRAQ with different shotgun proteomic methods in samples from an animal model of CAC that used repeated oral administration of dextran sulfate sodium (DSS) to induce CAC. As previously reported, iTRAQ protein quantification analysis identified fibrinogen beta, prohibitin, transgelin, Hsc-70-interacting protein, suppression of tumorigenicity 13

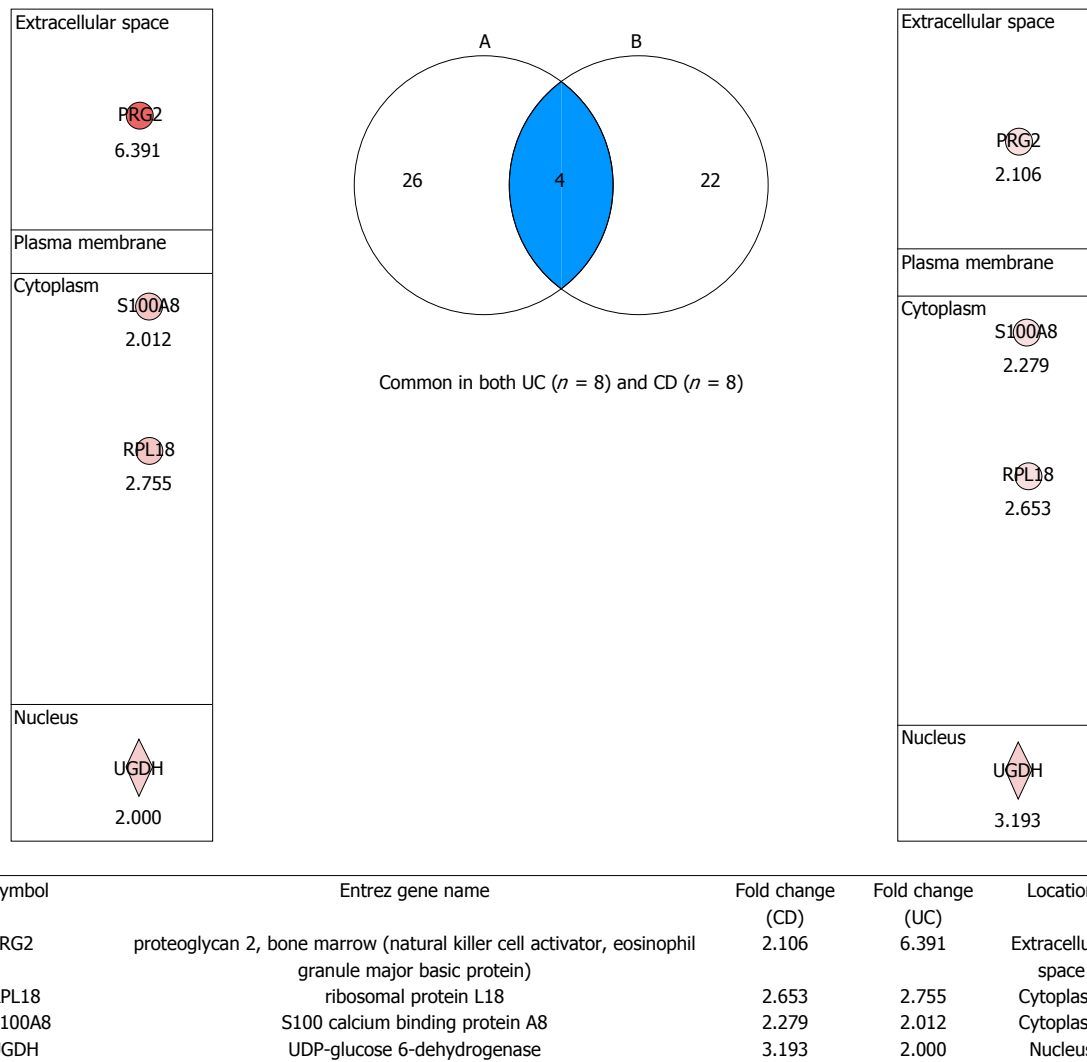


Figure 3 Proteomic markers for colitis-associated cancer risk in patients with both ulcerative colitis and Crohn's disease. After analyzing signaling pathways from label-free quantitative analysis, four important proteome biomarkers were identified: proteoglycan 2 (PRG2), S100 calcium binding protein A8 (S100A8), ribosomal protein L18 (RPL18), and UDP-glucose dehydrogenase (UGDH), all of which showed fold changes. Validation is ongoing to investigate these biomarkers for predicting colitis-associated cancer risk in patients with inflammatory bowel disease. UC: Ulcerative colitis; CD: Crohn's disease.

(ST13), a TKL kinase of the MLK family, dual leucine zipper kinase (MKL1), actin, beta, protein-coding gene (*ACTB*), ubiquitin carboxyl-term, esterase L3 (UCHL3), coronin, actin binding protein, 1A (CORO1A), hypoxanthin-guanine phosphoribosyltransferase (HPRT), glutathione peroxidase 1 (GPX1), estrogen receptor 1 (ESR1), transcriptional repressor protein 1 (YY1), transcription activator1, ATP-dependent helicase SMARCA4 (BRG1), brahma gene (BRM) and ornithine aminotransferase (OAT) as potential biomarkers for CAC in the DSS-induced colitic cancer model^[43].

CONCLUSION

MALDI imaging mass spectrometry (IMS) is a new technology to analyze small peptides in various samples and is a novel tool for studying molecular mechanisms in biological tissues. Recent studies have demonstrated considerable diagnostic and prognostic value, which should be

applicable to clinical settings in the near future^[81,82]. However, one challenge associated with the use of MALDI-IMS in the identification of potential biomarkers involves the systematic identification of peptides introduced in the MALDI matrix with association of top-down and bottom-up analysis^[83]. IMS will be investigated without target-specific reagents to identify new markers for the diagnosis, treatment, and prognosis of CAC, as well as the determination of effective therapies. In the near future, an era of tailored medicine will provide for diagnostic algorithms that include molecular parameters for the detection of early disease and treatment algorithms guided by predicting the individual course of the disease. However, more trials focused on discovering proteomic biomarkers will be necessary to guide the treatment of IBD with more advanced levels of biologicals or molecular targeted therapeutics for inflammation. Using label-free quantification methods on biopsied tissue from patients with IBD, four potential biomarkers, PRG2, S100A6 (calcy-

clin), RPL18, and UGDH, have been discovered. Further validation of these potential biomarkers will be necessary to ascertain their clinical value.

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Pulmonary manifestations of inflammatory bowel disease

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Abstract

Extraintestinal manifestations of inflammatory bowel disease (IBD) are a systemic illness that may affect up to half of all patients. Among the extraintestinal manifestations of IBD, those involving the lungs are relatively rare and often overlooked. However, there is a wide array of such manifestations, spanning from airway disease to lung parenchymal disease, thromboembolic disease, pleural disease, enteric-pulmonary fistulas, pulmonary function test abnormalities, and adverse drug reactions. The spectrum of IBD manifestations in the chest is broad, and the manifestations may mimic other diseases. Although infrequent, physicians dealing with IBD must be aware of these conditions, which are sometimes life-threatening, to avoid further health impairment of the patients and to alleviate their symptoms by prompt recognition and treatment. Knowledge of these manifestations in conjunction with pertinent clinical data is essential for establishing the correct diagnosis and treatment. The treatment of IBD-related respiratory disorders depends on the specific pattern of involvement, and in most patients, steroids are required in the initial management. Corticosteroids, both systemic and aerosolized, are the mainstay therapeutic approach, while antibiotics must also be administered in

the case of infectious and suppurative processes, whose sequelae sometimes require surgical intervention.

Key words: Inflammatory bowel diseases; Crohn's disease; Ulcerative colitis; Lung diseases

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Core tip: The clinicopathological patterns of pulmonary involvement in inflammatory bowel disease (IBD) consist of airway disease, lung parenchymal disease, thromboembolic disease, pleural diseases, enteric-pulmonary fistulas, and pulmonary function test abnormalities. The treatment of IBD-related respiratory disorders depends on the specific pattern of involvement, and in most patients, steroids are required in the initial management. This review focuses on the pulmonary manifestations of IBD in an attempt to avoid further health impairment and to alleviate symptoms by prompt recognition and treatment.

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INTRODUCTION

Inflammatory bowel diseases (IBDs) are chronic inflammatory diseases of unknown etiology that commonly involve the gastrointestinal tract^[1]. Crohn's disease (CD) and ulcerative colitis (UC) are the two main forms of chronic IBD. Extraintestinal and systemic manifestations occur commonly in patients with IBD (21%-41%)^[2-4], increase with duration of intestinal disease, and affect most organ systems^[5]. The extraintestinal manifestations are a significant cause of morbidity and may be particularly distressing for the patient^[6]. Extraintestinal manifestations are more common in CD and may include cutane-

ous (pyoderma gangrenosum and erythema nodosum), ocular (anterior uveitis and episcleritis), hepatic (pericholangitis and fatty liver), and articular (peripheral and axial arthropathies) diseases^[7]. Mouth ulcers and venous thrombosis also occur^[8]. In contrast, pulmonary involvement is rare^[9,10].

A possible link between UC and respiratory disease was described first by Turner-Warwick^[11] in 1968, but it was not until the work of Kraft *et al.*^[12] in 1976 that respiratory involvement came to be included in the list of established complications of IBD. The authors described six adult patients with IBD who developed chronic bronchial suppuration with or without bronchiectasis. Following this report, many investigators described a similar pattern, and other manifestations of pulmonary involvement were described, including: interstitial pneumonitis, panbronchiolitis, bronchiolitis obliterans organizing pneumonia (BOOP), inflammatory tracheal stenosis, serositis, pulmonary vasculitis, apical fibrosis, Langerhan's cell histiocytosis, sarcoidosis, and conditions resembling Wegener's granulomatosis^[13-23]. Respiratory diseases occurring in IBD may consist merely of a subclinical abnormal lung function or, in contrast, they may manifest as clear interstitial lung disease^[15].

Pulmonary alterations are often overlooked, especially when respiratory symptoms are already present before the diagnosis of IBD. The true prevalence of lung involvement in IBD remains unknown and it seems rather variable, because in some series only a few cases of respiratory complications have been found^[24]. However, it can be difficult to establish a relationship between respiratory diseases and IBD in patients who are already affected with pulmonary disease at diagnosis of IBD, or who are current smokers.

An ongoing bowel inflammation is not a prerequisite for the onset of respiratory alterations, because broncho-pulmonary diseases that develop after colectomy have been reported^[25]. Pulmonary abnormalities in IBD can present years after the onset of the bowel disease and can affect any part of the lungs. These may be overt or subclinical and do not correlate with the duration of IBD. The pulmonary manifestations are variously reported as occurring frequently during active disease, independent of disease activity, and even in post-colectomy patients^[26,27].

The pathogenesis of IBD causing lung abnormalities involves some of the following mechanisms: both the colonic and respiratory epithelia share an embryonic origin from the primitive foregut and have columnar epithelia with goblet cells and submucosal mucus glands; the lungs and gastrointestinal tract contain submucosal lymphoid tissue and play crucial roles in host mucosal defense^[28,29]. The similarity in the mucosal immune system causes the same pathogenic changes that may result from epithelial exposure to common antigens by inhalation and ingestion, leading to sensitization of the lymphoid tissue and inflammation^[13]. The activated inflammatory cells in the bowel tissues are capable of producing several circulating cytokines such as interleukin (IL)-1, IL-2 and IL-6 and tumor necrosis factor (TNF)- α . These and other media-

tors can regulate the endothelial cell adhesion molecules, alter leukocyte migration, increase production of damaging reactive oxygen metabolites, and induce damage of lung parenchyma^[6,30,31].

Although pulmonary involvement is well described in the literature, the evaluation and treatment of pulmonary disease associated with IBD remain a problem. The pulmonary associations of IBD are poorly characterized and early recognition is important^[32]. Here, we review the pulmonary manifestations that are associated with IBD.

PULMONARY DISEASES ASSOCIATED WITH IBD

Airway diseases

Airway disease from the trachea to the bronchioles has been reported in association with IBD^[29,33-50]. IBD was four times more prevalent among patients with airways disease compared with published local IBD prevalence in a retrospective analysis of outpatients over a 10-year period^[51]. The pathogenesis of IBD-related airway disease is unknown, but it is clearly inflammatory in nature. Severe tracheal inflammation and obstruction are rare manifestations of IBD and correspond to the presence of irregularly friable and hemorrhagic tissue at endoscopy^[52]. The tracheal epithelium is often ulcerated and is replaced by a thin layer of fibrin. The main symptoms are coughing, dyspnea, stridor and hoarseness^[42]. Upper airway involvement comprises glottic/subglottic stenosis, tracheal inflammation and stenosis^[13,40,42,43]. Most of this rare entity involves the trachea, presenting with shortness of breath, dysphonia, and cough^[45,46,53]. It can often be identified by history, complemented by a clear X-ray film and obstructive pattern on pulmonary function testing. Laryngoscopic evaluation is necessary because airway compromise can occur. The mucosa may exhibit a cobblestone appearance similar to that seen in affected intestines^[54]. Chest radiographs and computed tomography (CT) may show narrowing of any portion of the trachea, with circumferential tracheal wall thickening on CT^[27]. No predilection seems to exist for specific gender or type of bowel disease.

Large airway disease, strongly associated with UC^[18], is the most common presentation of pulmonary manifestations. Bronchial inflammation and suppuration are the most common manifestations of pulmonary involvement in IBD and include chronic bronchitis and bronchiectasis in which bronchial dilatation is visualized on chest X-ray or CT scan. Bronchiectasis is the most commonly reported entity and is noted in 66% of cases of IBD involving the large airways^[13,34,35,39,50]. Infrequently, in IBD patients developing new, persistent and unexplained symptoms of respiratory disease, particularly chronic productive cough, the presence of bronchiectasis may be demonstrated^[18]. The majority of patients with bronchiectasis have UC. IBD is inactive in many cases and curiously, in 60% of patients, the symptoms develop a few days to a few weeks following colectomy^[39,42,55]. The second most common

large airway disease in IBD is chronic bronchitis, which is distinguished from bronchiectasis only by the degree and extent of pulmonary abnormality^[56], and further abnormalities include suppurative large airway disease and acute bronchitis. The main symptom is chronic cough with purulent sputum poorly responsive to antibiotics^[57]. Bronchial biopsy shows similar features: squamous cell metaplasia in the mucosa that is sometimes infiltrated by neutrophils and a dense cuff of lymphocytes and plasma cells infiltrating the submucosa^[8].

Clinically, small airway disease is less frequently reported and is described as occurring in isolation from large airway disease. However, the recent advent of high-resolution CT has increased the detection of small airway involvement in these patients. These abnormalities seem to occur earlier in the course of the disease and at a younger age than large airway disease^[41,47-49,58-60]. Moreover, small airway disease is more frequently apparent before the onset of IBD than other airway diseases^[48]. CT shows bronchiolar wall thickening, mucoid impaction, centrilobular ground-glass nodules, and mosaic attenuation because of air trapping, and some patients have normal pulmonary function test (PFT) findings^[32].

Bronchiolitis is an inflammatory and potentially fibrosing condition affecting mainly the intralobular conducting and transitional small airways^[61]. Among small airway diseases are associated with IBD, bronchiolitis is the most frequently detected^[62,63]. Chronic bronchiolitis contributes to morbidity and/or mortality if it persists and/or progresses to diffuse airway narrowing and distortion or complete obliteration. Bronchiolitis in specific settings leads to bronchiolectasis, resulting in bronchiectasis. The main symptoms of bronchiolitis associated with IBD include mild productive cough and chronic bronchorrhea; wheezes are heard at auscultation. Small airway involvement can precipitate abnormalities on PFTs. Histological samples show varied patterns ranging from nonspecific fibrosing and stenosing bronchiolitis to an inflammatory lesion indistinguishable from the original description of panbronchiolitis^[8]. The CT appearances coupled with the evaluation of pulmonary function parameters usually lead to the diagnosis.

In IBD-related large airways disease, steroid drugs are effective, but recommendations for their use including dosage, duration and route of administration remain empirical. Steroids are the major therapy although some patients do not require systemic therapy. Clinical improvement with inhaled steroids alone or in combination with systemic steroids has been reported^[8,29,43,64]. Ineffectiveness of inhaled corticosteroids may be due to airways filled with inspissated secretions, in which case either topical corticosteroids via bronchoalveolar lavage (BAL) or systemic corticosteroids are recommended^[57]. Broadly speaking, inhaled steroids seem more effective and are better tolerated than oral steroids. Rarely, other forms of immunomodulation have been used to treat IBD-related airway disease^[65]. Small airway disease is usually refractory to inhaled steroids, and the improvement brought about by oral steroids ranges from slight to modest^[8]. Lung

transplantation has been required in some cases. Surgery of the colon, which may aggravate prior airway disease^[37], is not recommended for treatment of airway disease.

Lung parenchymal diseases

Lung parenchymal disease associated with IBD is relatively uncommon. Analysis of diffuse lung disease in IBD patients is further confounded by documented pulmonary sequelae to various medical therapies used to treat IBD. In contrast to other extraintestinal manifestations, lung parenchymal disease associated with IBD is seen more commonly with UC than CD^[8]. Age of onset varies, and there is a slight female predominance.

BOOP is the most commonly reported parenchymal manifestation of IBD^[63,66-69]. BOOP is often caused by inhalation injury, or results from a post-infection origin or drugs and may present acutely or subacutely with fever, cough, dyspnea and pleuritic chest pain^[15,70,71]. Chest radiography shows focal to diffuse peripheral predominant airspace opacities. CT shows scattered, nonsegmental, unilateral, or bilateral foci of consolidation, ill-defined centrilobular nodules, and large irregular nodules. It can be associated with other autoimmune diseases such as rheumatoid arthritis, lupus and Wegener's granulomatosis. Dyspnea and cough are the most common presenting symptoms. Systemic steroids are recommended for treatment but BOOP may also remit without treatment in a minority of cases^[13].

Other forms of parenchymal disease that may be related to IBD or drug toxicity are eosinophilic pneumonia and nonspecific interstitial pneumonitis. Although interstitial disease most commonly involves drug-induced reactions with mesalamine and sulfasalazine, a small number of unrelated cases of fibrosing alveolitis and eosinophilic pneumonia have been reported^[72-77]. On CT, peripheral consolidation predominates in cases of eosinophilic pneumonia, whereas nonspecific interstitial pneumonitis shows ground-glass opacities, interlobular septal thickening, and irregular linear opacities^[78]. The interstitial lung infiltrates have been proven histologically to be either pulmonary vasculitis^[79-81] or more often granulomatous disease^[82-85].

Pulmonary nodules have been infrequently reported in patients with IBD. Histologically, these lesions have been reported to be necrobiotic, granulomatous, or otherwise^[86]. Necrobiotic nodules, composed of sterile aggregates of neutrophils with necrosis, may also be seen in rheumatoid arthritis, Wegener's granulomatosis, or septic pulmonary emboli, and should be differentiated from malignancy and infection. An infectious origin should be excluded because necrobiotic nodules will respond to steroids but not to antibiotics. Sarcoidosis and CD are both granulomatous diseases, of the lung and bowel, respectively. It is not surprising that these two diseases may simultaneously appear in the same patient, with pulmonary involvement^[87], even though this happens rarely and the two diseases usually follow an independent clinical course^[88]. An infectious cause, specifically atypical *Mycobacterium* has been postulated to contribute to granuloma

formation in both sarcoidosis and CD, and has even been detected in tissues from patients with both diseases^[89].

The manifestations of lung parenchymal disease in IBD usually respond dramatically to inhaled and/or systemic steroids. Steroids administered orally lead to marked improvement in patients with interstitial lung disease, BOOP, pulmonary infiltrates with eosinophilia, and necrotic nodules. Intravenous steroids are required in the initial management of life-threatening complications such as extensive interstitial lung disease. The addition of cyclophosphamide or infliximab may show rapid clinical and radiological response and are well tolerated in some cases^[90,91].

Thromboembolic diseases

IBD is a chronic inflammatory condition, characterized by microvascular and macrovascular involvement. Inflammation and immune response could lead to endothelial dysfunction, which is the earliest stage of the atherosclerotic process^[92]. Chronically inflamed intestinal microvessels of IBD patients have demonstrated significant alterations in their physiology and function compared with vessels from healthy and uninvolved IBD intestine^[93]. Thromboembolism is an extraintestinal manifestation and an important cause of mortality in IBD^[94]. The incidence of thromboembolic events in IBD patients is three to four times higher than in age-matched control subjects^[95,96]. It happens at an earlier age than in non-IBD patients. The majority of thromboembolic events among IBD patients are venous thromboembolism, manifested as either deep venous thrombosis or pulmonary embolism, but arterial thromboembolism and venous thrombosis at unusual sites have also been reported^[97]. Prothrombotic risk factors in IBD patients could be distinguished as acquired, such as active inflammation, immobility, surgery, steroid therapy, and use of central venous catheters, and inherited^[93].

The risk of thromboembolism appears to be multifactorial and related to mucosal inflammatory activity in most patients. Pulmonary embolism should be always considered in IBD patients with breathing difficulties. However, the diagnosis of venous and arterial thromboembolism is extremely challenging and requires a high degree of vigilance. Deep vein thrombosis and pulmonary embolism may be clinically silent or manifest with only a few specific symptoms. Up to one-third of thromboembolic events in this population occur while IBD is quiescent, suggesting an unknown risk factor that is unrelated to treatment or disease activity^[13]. The pathogenesis of increased thrombotic risk among patients with IBD is unclear. About 80% of IBD patients have active disease when pulmonary embolism occurs^[98]. Early diagnosis plays a central role in optimizing the therapeutic intervention and reducing the risk of short-term and long-term thrombosis-associated complications. The decision regarding the duration of systemic anticoagulation must take into account the individual risk of intestinal bleeding^[99].

Pleural diseases

Rarely, IBD involves the pleural space and pericardium,

causing inflammatory exudative pleural and/or pericardial effusions^[100,101]. This is a relatively rare presentation of the uncommon and probably under-reported and under-recognized pulmonary extraintestinal manifestations of IBD^[102]. Pleuropericardial inflammatory disease and effusion can be directly related to IBD, its complications, associated infections, or the medications used to treat it^[103]. Most patients are young, male, and have UC during the quiescent phase of the disease. The manifestations of pleural disease can be classified as: pneumothorax^[104], pleural thickening^[105], pleuritis, and pleural effusion^[106]. Pleural fluid directly related to IBD is usually unilateral, an exudate with neutrophils, and may be hemorrhagic. Mesalazine may also induce lupus-like symptoms, such as arthralgia, pericarditis, tamponade, and/or pleural effusion, with positive antinuclear antibody^[107]. It is important to evaluate pleural effusion and rule out other etiologies before making this diagnosis. Pleural or pericardial biopsies are rarely necessary, and probably show nonspecific acute and chronic inflammatory changes^[103]. Although the specific pathophysiology of pleuropericardial disease in patients with IBD remains unclear, the response to systemic steroids is usually adequate. However, pleural drainage may be required occasionally.

Enteric-pulmonary fistulas

Fistula formation is frequent in CD and occurs in 33% of patients^[107]. Most of the fistulas appear in the perineal area^[108]; to date, only a few reports (mostly as single cases) are available on the occurrence of enteric-pulmonary fistulas in IBD, such as colobronchial^[109-112], ileobronchial^[113], and esophagobronchial^[114,115] fistulas. In most cases, colobronchial fistulas extend from the splenic flexure in the colon to the lower lobe of the left lung. This is likely due to the anatomical proximity between the two structures. However, Mercadal *et al.*^[116] reported a rare case of right-sided colobronchial fistula in a 47-year-old, severely malnourished man with a history of regional enteritis and recurrent right lower and middle lobe pneumonia, medically managed with the addition of the immunomodulator infliximab prior to surgery.

Diagnosis of fecopneumothorax is based on meticulous clinical examination and additional diagnostic procedures. Recurrent pneumonia with feculent sputum in patients with CD should raise suspicion of colobronchial fistula. Once the abnormal connections between the bowel and respiratory tract are suspected, an enema using water-soluble contrast medium certainly helps to confirm the presence of fistulas^[110]. Abdominal and thoracic CT scan or magnetic resonance imaging could provide additional information about the stage of the disease and exclude the presence of abscess or fluid collection in the abdominal cavity. Colopleural fistula and fecopneumothorax are rare but life-threatening complications of CD^[117]. Surgical treatment is mandatory as soon as the diagnosis is established^[118].

Pulmonary function test abnormalities

PFT abnormalities are found frequently in patients with

IBD without presence of any respiratory symptoms and lung radiograph findings^[20]. IBD patients show significantly decreased lung function tests in comparison to healthy controls. In a review including over 600 patients with UC, more than 50% of patients showed abnormal PFT results when compared to healthy controls, and the decrease in diffusion capacity of the lung for carbon monoxide (DLCO) was the most common defect^[15]. Various studies testing pulmonary function in patients with IBD have revealed a spectrum of abnormalities including restrictive disease, obstructive disease, bronchial hyperresponsiveness and hyperinflation as well as a decreased diffusion capacity of the lung^[119-133]. The severity and frequency of these PFT abnormalities that are detected even in the remission periods increase with disease activity. There is no difference between UC and CD in PFTs^[51], and smoking status is not predictive of these abnormalities.

The most commonly described abnormality is a decrease in lung diffusion capacity^[66]. In most studies, this alteration could not be predicted by current or past smoking status, occupational history, or current medication use. Lung transfer factor for carbon monoxide abnormalities is related to the degree of disease activity^[122]. Pulmonary involvement in IBD is often asymptomatic and detectable only at the time of lung function investigation. This is further supported by the finding of Wallaert *et al*^[134] of a high proportion of latent lymphocytic pulmonary alveolitis in the BAL of 18 consecutive patients with CD; all free from respiratory symptoms and showing normal chest X-ray. Therefore, PFT may be used as a noninvasive diagnostic procedure in determining the activation of IBD and might aid early diagnosis of latent respiratory involvement^[135]. Early recognition is important, because PFT abnormalities can be steroid responsive^[136].

PFT studies in IBD suggest that subclinical pulmonary disease may be present in a large subpopulation of patients. A high degree of suspicion is necessary to detect the pulmonary abnormalities in IBD, because a large proportion of symptom-free patients have abnormal findings on pulmonary function testing. Although the mechanism of these abnormalities remains unclear, it may be a result of the increased capacity of alveolar macrophages to produce superoxide anions, which has been shown in some patients with CD^[137].

DRUG-RELATED LUNG DISEASES

Although drug-related diseases are not “proper” IBD-associated diseases, because IBD patients use several drugs for prolonged periods of time, it is not surprising that some of these may also cause problems to the lungs. Therefore, this type of pathology must be kept in mind for patients taking azathioprine (AZA), 6-mercaptopurine (6-MP), sulfasalazine, mesalamine, methotrexate, and anti-TNF- α .

AZA and 6-MP

AZA and 6-MP are therapeutic options for patients with moderate to severe IBD^[138]. However, between 10% and

29% of patients treated with these drugs are forced to stop therapy due to side effects. Pulmonary toxicity due to these drugs has been reported infrequently in the literature, although interstitial pneumonitis^[139], BOOP^[140], chronic pneumonitis/fibrosis and pulmonary edema^[141] have been described after use of AZA and 6-MP. Although rare, AZA and 6-MP can cause direct, dose-dependent and serious pulmonary toxicity^[140,142]. The largest number of cases of lung toxicity related to AZA was described in seven patients undergoing renal allograft transplant immunosuppression with AZA^[142]. Lung biopsies revealed interstitial pneumonitis in 5 patients and diffuse alveolar damage in 2; 3 patients died and the other 4 improved after stopping AZA, and in 2 of these patients, cyclophosphamide therapy was needed to resolve this side effect completely. Thus, it is important for clinicians to have a high index of suspicion for this adverse reaction, which occurs within 1 mo after purine analog use in IBD.

Sulfasalazine and mesalamine

Sulfasalazine and mesalamine are commonly used medications for the long-term treatment of IBD, and their side effects may be dose-related or idiosyncratic and should be differentiated from the respiratory involvement occurring in IBD and due to the underlying disease, although this is challenging because they share similar pathological features^[47]. Commonly reported lung pathology related to the use of these compounds is mostly due to interstitial disease^[54,128,143-145], although eosinophilic pleuritis^[146], eosinophilic pneumonia^[71,147-150], and bronchiolitis obliterans^[67] have also been described. Patients present with progressive respiratory symptoms such as dyspnea, chest pain and cough, and radiographic abnormalities. Alternatively, sulfasalazine and mesalamine may induce asymptomatic lung injury more commonly than is presently suspected^[151]. Although sulfasalazine or mesalamine-induced lung injury is a rare entity, its possibility should be fully considered in patients developing unexplained respiratory symptoms while on sulfasalazine or mesalamine therapy^[150]. In most cases, symptoms appear after 2-6 mo of drug use, whereas in a few cases they appear after a few days or after many years. These pulmonary toxicities appear reversible after withdrawal of the drug, and in some cases, with the use of systemic corticosteroids^[152,153].

Methotrexate

Methotrexate (MTX) may be useful in the treatment of IBD^[154], but can cause adverse effects in the lungs, which in some cases are lethal^[155]. The mechanism of MTX-induced lung pathology remains unclear. A hypersensitivity reaction was suggested by lung biopsy findings: interstitial pneumonitis, granuloma formation and bronchiolitis^[156], and by BAL findings: lymphocytic alveolitis, increased eosinophils and reversed CD4/CD8 ratio^[157], together with the clinical findings of fever, peripheral eosinophilia and response to corticosteroids. MTX may also cause pneumonitis^[158], and abnormal ventilation is an early sign and should lead to further investigation^[159]. The diagnosis of

MTX-induced lung disease is difficult because there are no pathognomonic findings and this condition may mimic other pulmonary diseases. The most frequent complaints include dyspnea, fever and nonproductive cough. PFTs show a restrictive picture with low CO diffusion capacity. MTX-related lung toxicity is potentially fatal, thus, regular monitoring of the status of the respiratory system in MTX-treated patients is necessary and patients should be instructed to report any new pulmonary symptoms without delay^[160]. Besides supportive therapy, withdrawal of MTX seems to be a logical approach.

Biological therapy

Biological therapy with anti-TNF drugs such as infliximab, adalimumab and certolizumab has represented a significant advance in the treatment of IBD over the past few years^[161-163]. However, serious side effects do occur, necessitating careful monitoring of therapy^[164]. Several associated opportunistic infections have been observed as a result of suppression of T-cell-mediated immunity; the most frequent being tuberculosis^[165-167]. Physicians should be aware of the increased risk of reactivation of tuberculosis in patients treated with anti-TNF agents and regularly look for usual and unusual symptoms of tuberculosis. Moreover, the use of biological therapy has been associated with *Pneumocystis carinii* pneumonia^[168], as well as with other pulmonary infections (coccidiomycosis, histoplasmosis, aspergillosis, nocardia asteroides, actinomycosis and listeriosis)^[169-173], especially in older patients^[174].

Although infective complications are the most feared after the use of biological agents, these may induce other uncommon effects in the lung, such as acute respiratory distress syndrome^[175], diffuse alveolar hemorrhage^[176], non-bronchiolitis inflammatory nodular pattern of the lung^[177], and interstitial lung disease^[178-180]. Close observation of patients undergoing treatment with TNF inhibitors for evolving signs and symptoms of autoimmunity is required. Organ involvement is unpredictable, which makes correct diagnosis and management extremely challenging.

CONCLUSION

Pulmonary manifestations of IBD are being increasingly recognized. The involvement of the respiratory system in IBD, which can range from a simple defect of pulmonary function without symptoms, to fibrosing alveolitis with a greater risk of mortality, is relatively rare but sometimes potentially harmful. Early identification of latent pulmonary involvement is important to prevent future and more severe respiratory impairment and can be life-saving. The manifestations in the lung vary and often represent a confounding diagnostic problem. It is imperative for clinicians to maintain a high index of suspicion for the development of pulmonary disease in the setting of IBD in order to institute appropriate treatment early and avoid further complications and morbidity in IBD patients, and to recognize prompt treatment for these events. Steroids are effective in the majority of cases.

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Inflammatory bowel disease and thromboembolism

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Abstract

Patients with inflammatory bowel disease (IBD) have an increased risk of vascular complications. Thromboembolic complications, both venous and arterial, are serious extraintestinal manifestations complicating the course of IBD and can lead to significant morbidity and mortality. Patients with IBD are more prone to thromboembolic complications and IBD *per se* is a risk factor for thromboembolic disease. Data suggest that thrombosis is a specific feature of IBD that can be involved in both the occurrence of thromboembolic events and the pathogenesis of the disease. The exact etiology for this special association between IBD and thromboembolism is as yet unknown, but it is thought that multiple acquired and inherited factors are interacting and producing the increased tendency for thrombosis in the local intestinal microvasculature, as well as in the systemic circulation. Clinicians' awareness of the risks, and their ability to promptly diagnose and manage thromboembolic complications are of vital importance. In this review we discuss how thromboembolic disease is related to

IBD, specifically focusing on: (1) the epidemiology and clinical features of thromboembolic complications in IBD; (2) the pathophysiology of thrombosis in IBD; and (3) strategies for the prevention and management of thromboembolic complications in IBD patients.

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Thrombosis; Thromboembolism; Hypercoagulability; Epidemiology; Endothelial dysfunction; Treatment

Core tip: Thromboembolic complications, both venous and arterial, are serious and challenging complications of inflammatory bowel disease (IBD) and can lead to significant morbidity and mortality. Thrombosis is a specific feature of IBD that can be involved in both the occurrence of thromboembolic events and the pathogenesis of the disease itself. The cause for this association between IBD and thromboembolism is as yet unknown, but multiple acquired and inherited factors have been implicated. Clinicians' awareness of the risks, and knowledge about the diagnosis and management of thromboembolic complications are of vital importance.

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INTRODUCTION

Thromboembolic events, both venous and arterial, are serious extra-intestinal manifestations complicating the course of inflammatory bowel disease (IBD) and can lead to significant morbidity and mortality. The increasing evidence that patients suffering from both Crohn's dis-

ease (CD) and ulcerative colitis (UC) are more prone to thromboembolic complications compared to the general population implicates the IBD *per se* as a risk factor^[1,2]. Moreover, recent data support the theory that thrombosis and thromboembolism are disease-specific manifestations in IBD, and that they also may be contributing factors in the pathogenesis of the luminal disease. In this review we discuss how thromboembolic disease is related to IBD, specifically, the following: (1) the epidemiology and clinical features of thromboembolic complications in IBD; (2) the pathophysiology of thrombosis in IBD; and (3) strategies for the management, prevention and treatment, of thromboembolic complications in IBD patients.

IBD AND THROMBOEMBOLIC COMPLICATIONS

Historically, in 1936, Barger and Barker^[3] first reported arterial and venous thrombotic complications in UC patients. In a large Mayo Clinic survey, Talbot *et al*^[4] found that 1.3% of their IBD patients manifested thromboembolic complications, while other early studies reported an even higher incidence, up to 7%^[5-8]. Moreover, an important and interesting observation came from autopsy studies which found a much higher incidence of venous thromboembolic complications, up to 39%, in UC patients, implying that most of the thromboembolic episodes were either not clinically overt or were overlooked. The thromboembolic complications were the third leading cause of death (10%) in those patients^[9].

Thrombosis occurs more often in the deep veins of the legs and the pulmonary circulation^[4]; however, arterial thromboembolic complications (ATEs) and numerous other less frequent sites of venous thrombosis have also been described, including cerebrovascular (CVA) disease^[10,11], internal carotid artery occlusion^[12], mesenteric and portal vein thrombosis^[13], Budd-Chiari syndrome^[14], cutaneous gangrene secondary to microvascular thrombosis^[15], retinal vein occlusion^[16-18], and ischemic heart disease (IHD)^[19].

Venous thromboembolism

Over the past decade in particular, many large case-controlled and cohort studies, in centers throughout the world, have focused on defining the association of IBD with the risk of venous thromboembolism (VTE) and have contributed to significant progress in clarifying the epidemiological and the clinical features of VTEs in IBD^[1,2,20].

Some assessed the incidence and the risk of VTE in IBD patients compared to the general population^[21-25], while others evaluated the risk of VTE in hospitalized IBD patients compared to hospitalized non-IBD patients^[26-30]. A few studies were more focused, and analyzed the risk of VTE in pregnant females with IBD^[31,32], the risk of VTE in postoperative IBD patients^[33,34] and, finally, one study evaluated the risk of recurrent DVT in adult IBD patients^[35].

The overall risk of VTEs, deep vein thrombosis (DVT) and pulmonary embolism (PE), in IBD patients has been estimated in two recent meta-analyses^[1,36]. Despite the heterogeneity and the limitations of the studies included, both meta-analyses revealed an approximately 2-fold increased risk for VTEs in IBD patients. Yuhara *et al*^[36], reported that the overall relative risk (RR) for DVT and PE in patients with IBD compared to subjects without IBD was 2.20 (95%CI: 1.83-2.65), and Fumery *et al*^[1] reported that the overall risk of VTE in IBD patients was increased by 96% compared to the general population (RR = 1.96; 95%CI: 1.67-2.30) with no differences between CD and UC patients.

In a recent nationwide multicenter study conducted in Austria, Papay *et al*^[37] investigated the prevalence and the incidence of VTEs in 2811 IBD patients and described many related clinical features. The overall prevalence of all VTEs was 5.6% (157/2811) and the incidence of all VTEs was 6.3/1000 person years. The majority of VTEs were DVT and/or PE (about 90%; 142/157), while other locations of venous thrombosis were rare (about 10%; 15/157) including the portal, the superior mesenteric, the splenic, the internal jugular, and the cerebral veins. No difference was found between CD and UC for the frequency of all VTEs, although the prevalence and incidence for DVT and/or PE was a little higher in CD patients.

VTEs occur earlier in life in IBD patients than in non-IBD thrombotic patients^[21,26,38,39]. Bernstein *et al*^[21] analyzed data from IBD and non-IBD hospitalized patients and found that the risk of VTE, DVT and/or PE, was overall higher in hospitalized IBD patients. The most striking difference in the risk was observed in patients who were less than 40 years of age, with an incidence rate ratio (IRR) for VTE of 4.5 for UC and 9.6 for CD compared with the non-IBD patients.

Thromboembolic complications are a significant cause of morbidity and mortality in IBD patients^[39-42]. In the study by Talbot *et al*^[4], 25% of IBD patients with thromboembolic complications had a fatal outcome during the thrombotic episode. Recently, Nguyen and Sam^[27], also reported higher rates of VTEs in hospitalized IBD patients than in non-IBD hospitalized patients. As was the case in Bernstein's report, those who were less than 40 years of age were at the greatest risk. Hospitalized IBD patients with thrombosis had a greater in-hospital mortality risk when compared to hospitalized IBD patients without thrombosis (OR = 2.5; 95%CI: 1.83-3.43) and to non-IBD hospitalized patients with thrombosis (OR = 2.1; 95%CI: 1.6-2.9). In addition, the occurrence of VTEs in hospitalized IBD patients significantly increased the length of hospital stay and health resource utilization cost.

Patients with IBD are at increased risk for postoperative VTE^[43]. Merrill and Millham^[33] reported that IBD patients were at increased risk for developing postoperative DVT or PE compared to non-IBD patients, especially after non-intestinal surgery. Furthermore, Wallaert *et al*^[34] studied VTE during the first 30 postoperative days in a

Table 1 Incidence, risk and clinical features of venous thromboembolism in inflammatory bowel disease patients

Venous thromboembolism and IBD	
Prevalence:	1.3%-7% - postmortem about 40%
Risk overall:	about 2-3-fold
Features	
	Deep vein thrombosis (legs) and pulmonary embolism
	Younger age
	Spontaneously
	Recur - 30% (risk about 2.5-fold)
	Significant morbidity and mortality
Risk factors	
	Active disease (ambulatory and hospitalized patients)
	Complicated disease
	Corticosteroid use
	Extensive colonic involvement (UC and CD)
	Recent hospitalization
	Surgery
	Pregnancy
	Previous history of VTE
	Family history of VTE

IBD: Inflammatory bowel disease; VTE: Venous thromboembolism; UC: Ulcerative colitis; CD: Crohn's disease.

large cohort of IBD patients having colorectal surgery (10431 patients, 5001 with UC and 5430 with CD) and found an overall incidence of 2.3% for VTEs (242 VTEs; 178 DVTs and 46 PEs). The rates of VTEs were higher for UC patients compared to CD patients (3.3% *vs* 1.4% respectively). The thromboembolic episodes occurred at an average of 10 d postoperatively and were associated with significant morbidity and mortality.

Recurrence of thromboembolic events has been previously reported as being 10%-13% in IBD patients^[4,39]. Novacek *et al*^[35] reported an approximately 30% probability of VTE recurrence in IBD patients 5 years after discontinuation of the anticoagulant treatment for the first VTE. The risk for recurrent VTE was higher in IBD patients compared to non-IBD patients. IBD, irrespective of the activity status, was found to be an independent risk factor for recurrent VTE with a relative risk of 2.5 (95%CI: 1.4-4.2). In accordance with that, Papay *et al*^[37] reported a similar incidence of recurrent VTEs (25%) in IBD patients; in the majority, the VTEs occurred in the same location (70%) and were of the same type (DVT or PE) as with the first episode.

Thromboembolic events are more frequent during active phases of IBD and correlate with the extent and location of the disease; most of them occur without evidence of provoking factors^[4,22,40,44]. Complicated IBD (fistula, stenosis, abscess)^[22,27], use of corticosteroids^[24], and recent hospitalization for IBD^[24] were all associated with increased risk for VTEs. Solem *et al*^[40] reported that 80% of IBD patients (both CD and UC) had active disease at the time of VTE. Regarding the extent of disease in patients with VTE, 76% of the UC patients had pancolitis and 79% of the CD patients had colonic involvement. In contrast to the above, Talbot *et al*^[4] found that almost 30% of VTEs occurred when the disease was in remission and that 77% of the peripheral VTEs occurred

spontaneously. In a recent study, Grainge *et al*^[25] assessed the risk of VTE at various activity phases of IBD (flare, chronic activity, remission) in a retrospective cohort study of 13756 IBD patients and 71672 matched controls from the prospectively generated General Practice Research Database (United Kingdom). They found that there is a significantly increased overall risk of VTE in IBD patients compared to controls during all phases of IBD (HR = 3.4; 95%CI: 2.7-4.3). The risk was most prominently increased during a flare (HR = 8.4; 95%CI: 5.5-12.8) compared with periods of chronic activity (HR = 6.5; 95%CI: 4.6-9) and periods of clinical remission (HR = 2.1; 95%CI: 1.6-2). In this unique study, the overall relative risk of VTEs in ambulatory IBD patients compared to controls appeared to be higher than in hospitalized IBD patients compared to controls (HR = 4.3; 95%CI: 3.3-5.7 *vs* HR = 2.1; 95%CI: 1.4-3.2). This apparent difference in the risk between ambulatory and hospitalized IBD patients compared to controls was even higher during periods of disease flares (HR = 15.8 *vs* 3.2, respectively). However, when the data were expressed as absolute risk of VTEs per 1000-person years it was obvious that the hospitalized IBD patients were more prone to thrombosis (25.2) than the non-hospitalized IBD patients (1.8), especially during a flare of the disease (37.5 *vs* 6.4, respectively). Finally, the risk of VTEs was higher during flares and chronic activity periods compared to periods of remission of the disease, both in ambulatory and hospitalized IBD patients. These data are further supported by the recent study of Papay *et al*^[37] who reported that 77% of VTEs in the IBD cohort occurred spontaneously, 77% occurred in outpatients and 66% occurred during an active period of the disease.

Collectively, all the recent studies discussed above confirm that patients with both CD and UC are at an approximately two-fold increased risk for VTEs compared to the general population or to non-IBD patients. The VTEs, mainly DVT and/or PE, tend to occur spontaneously, at a younger age, and more frequently during periods of active disease, in both ambulatory and hospitalized patients. There is also increased risk during periods of remission, during pregnancy, and postoperatively. They also recur frequently after the first episode. The VTEs in IBD patients are associated with significant morbidity and mortality (Table 1).

Arterial thromboembolism

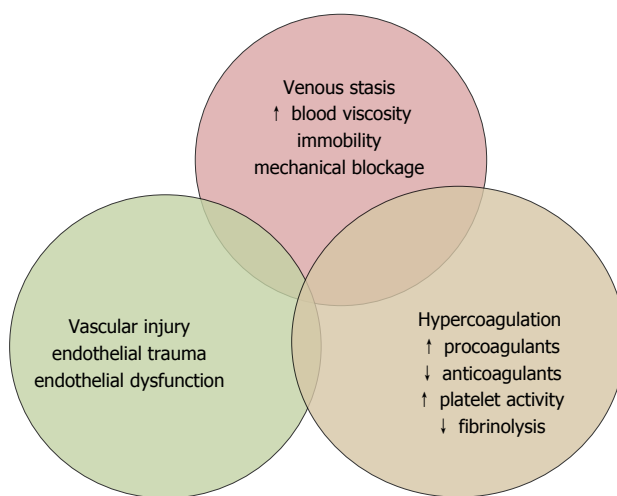
Numerous cases and case series have reported ATEs in IBD patients. In general, ATEs occur less frequently than VTEs in IBD patients, and may involve the thrombosis and/or occlusion of the cerebral^[11,16], retinal^[16,17], carotid^[12,45,46], coronary^[19], splanchnic^[47], iliac^[48,49], renal^[48,49], and limb (upper and lower)^[50,51] arteries or the aorta^[48,49,52]. They are more common after interventional or surgical procedures, but they can also occur spontaneously^[4,51].

Recent studies^[53-59] and a meta-analysis^[60] provide evidence for an association between IBD and ATE, similar to that which exists with VTEs, despite the fact there

Table 2 Incidence, risk and clinical features of arterial thromboembolism in inflammatory bowel disease patients

Arterial thromboembolism and IBD	
Common sites and risk	
Cerebrovascular events about 1.2-fold	
Ischemic heart disease about 1.2-fold	
Mesenteric ischemia about 3.5-fold	
Features	
Younger age	
Female	
Post-surgically >> spontaneously	
Active disease (ambulatory and hospitalized patients)	
Significant morbidity and mortality	

IBD: Inflammatory bowel disease.

**Figure 1** Basic mechanisms of thrombosis (Virchow's triad).

are some controversial findings in other studies^[30,61,62]. In their meta-analysis Singh *et al*^[60] analyzed data from 9 studies and found that IBD was associated with a modest increase for the risk of cardiovascular morbidity. In particular, 5 studies reported 2424 CVA events in 98240 patients with IBD and six studies reported 6478 occurrences of IHD in 123907 patients with IBD, which translates to a modest 18% increase in the overall risk for both CVA (adjusted OR = 1.18; 95%CI: 1.09-1.27) and IHD (adjusted OR = 1.18; 95%CI: 1.08-1.31). In addition, the risk was higher in females and young patients (age < 40-50 years). There were no differences between UC and CD patients. Finally, 2 studies reported 148 patients with peripheral arterial disease in 25559 patients with IBD, but the analyses showed that IBD was not associated with a significant increase for the risk of peripheral arterial disease (adjusted OR = 1.15; 95%CI: 0.96-1.38).

A nationwide United States study^[30], which investigated the association of cardiovascular diseases in 148229 hospitalized subjects with IBD compared to 17261952 controls, showed a significantly increased risk for mesenteric ischemia (adjusted OR = 3.4; 95%CI: 2.9-4.0) and thromboembolic disease in hospitalized IBD patients. Fumery *et al*^[11], in their meta-analysis, con-

cluded that overall IBD was associated with an increased risk of thrombovascular events. The major risks were for VTE and mesenteric ischemia and, to a lesser degree, for arterial thromboembolism and ischemic heart disease. Although they did not find an increase in the risk of cardiovascular mortality in IBD patients, Kristensen *et al*^[54], who investigated the risk of myocardial infarction (MI), stroke, and cardiovascular death in patients with IBD with correlation to disease activity in a nationwide Danish cohort, reported that the IBD is associated with increased risk of MI, stroke, and cardiovascular death during periods with active disease, including acute flares or persistent activity.

To summarize, recent data show that patients with IBD, both CD and UC, are at an increased risk for ATEs, mainly CVA, IHD and mesenteric ischemia, albeit to a lesser degree than for VTEs. The ATEs tend to occur spontaneously or post-surgically, at a younger age, in females, more frequently during periods of active disease and are associated with significant morbidity and mortality (Table 2).

MECHANISMS OF THROMBOSIS IN IBD

In contrast to hemostasis, which is a normal response to vascular injury, thrombosis is pathological coagulation occurring spontaneously or following a minimal vascular injury. The underlying cause of thrombosis is an imbalance between prothrombotic and antithrombotic mechanisms. The tendency towards thrombosis is related to three basic mechanisms, as defined by the Virchow's triad: vascular stasis, endothelial injury/vascular damage and hypercoagulability (Figure 1).

Evidence from the literature suggests that thrombosis is a specific feature of IBD that is involved in both the occurrence of thromboembolic events and the pathogenesis of the disease itself^[44]. Multifocal vascular infarcts in the intestinal microcirculation, characterized by chronic vasculitis, with focal arteritis and fibrin deposition, have been reported in patients with CD^[63]. Histological studies have also found mucosal capillary thrombi in patients with UC^[64]. In addition, Thompson *et al*^[65], in a large study involving 129 hemophilia centers in United Kingdom, reported a lower than expected incidence of IBD in 9562 patients with hemophilia or von Willebrand's disease and concluded that a congenital bleeding diathesis may have a protective role against the development of IBD. Furthermore, Miehsler *et al*^[22] demonstrated that IBD *per se* is a risk factor for thromboembolic and concluded that thromboembolism is a specific feature of IBD since neither rheumatoid arthritis, another chronic inflammatory disease, nor coeliac disease, another chronic bowel disease, had an increased risk of thromboembolism.

The exact etiology for the higher occurrence of thromboembolism in IBD and the specific association between them is yet unknown, though it seems that multiple acquired and inherited factors may be involved (Table 3).

General acquired prothrombotic factors such as

Table 3 Acquired and hereditary thrombotic risk factors in inflammatory bowel disease patients

Factors	Mechanism
Acquired	
Inflammation	Hypercoagulation, vascular endothelial injury
Immobilization	Stasis
Indwelling IV catheters	Vascular injury
Dehydration	Stasis
Steroid use	Hypercoagulation
Oral contraceptives	Hypercoagulation
Surgery	Stasis, hypercoagulation, vascular injury
Pregnancy	Stasis, hypercoagulation
Cancer	Hypercoagulation
Infections	Hypercoagulation
Age	Hypercoagulation
Smoking	Hypercoagulation
Hereditary	
Proteins C and S deficiencies	Hypercoagulation
Antithrombin deficiency	Hypercoagulation
Factor V Leiden	Hypercoagulation
Hyperhomocysteinemia-MTHFR gene mutation	Hypercoagulation
Prothrombin gene mutation G20210A	Hypercoagulation
Dysfibrinogenemia	Hypercoagulation

inflammation, older age, surgery, prolonged immobilization, central venous catheters, fluid depletion, steroid therapy, smoking, and oral contraceptives are frequently observed in IBD patients, but their presence cannot adequately explain the increased risk for thromboembolisms in IBD^[44]. On the other hand, many studies and reviews have failed to establish a significant association of the inherited thrombophilias, such as factor V Leiden, prothrombin G20210A mutation, MTHFR mutation-related hyperhomocysteinemia, protein C, S and antithrombin deficiencies, with the increased risk of thrombosis in IBD patients, although their co-existence with IBD has a synergistic role in thromboembolic complications^[44,66,67]. Multiple risk factors are often present in IBD patients^[40], although none of them is more significant than the others, it seems obvious that as more risk factors accumulate in a patient, thrombosis is more likely to occur in that patient.

Inflammation and hypercoagulation in IBD

Inflammation, both intestinal and systemic, is the prominent feature in IBD. Inflammation and thrombosis are probably interrelated in IBD, through complex and as yet not fully understood pathways. Consequently, local and systemic intravascular hypercoagulable and prothrombotic states or even frank thrombosis, may represent contributing underlying factors in IBD pathogenesis^[68].

The hypercoagulable state has been associated particularly with active disease^[69]. Several studies have reported abnormalities in various components of hemostasis and the coagulation cascade during exacerbations of IBD (Table 4), as follows: (1) elevated levels of coagulation factors (V, VIII, von Willebrand, and fibrinogen) and

Table 4 Prothrombotic abnormalities of hemostasis and coagulation in inflammatory bowel disease patients

Category	Abnormality
Coagulation factors	↑ V, VIII, vWf, and fibrinogen
Products of thrombin generation	↑ F1 + 2, TAT
Products of fibrin formation	↑ fibrinopeptide A, D-Dimers
Vascular endothelium activation	↑ vWf, thrombomodulin
Acquired deficiencies and dysfunction of natural anticoagulants	↓ protein C, protein S, and AT
Defects in fibrinolytic system	↓ t-PA ↑ PAI-1
Platelets	↑ number, activation and aggregation

vWf: von Willebrand; F1 + 2: Prothrombin fragment 1 + 2; TAT: Thrombin- antithrombin complex; t-PA: Tissue plasminogen activator; PAI-1: Plasminogen-activator inhibitor type-1.

products of thrombin and fibrin formation (fibrinopeptide A, prothrombin fragment 1+2 [F1+2], thrombin-antithrombin complex [TAT], and D-Dimers)^[70-74]; (2) increased markers of vascular endothelial activation (von Willebrand factor and thrombomodulin)^[74-78]; (3) acquired deficiencies and dysfunction of natural anticoagulants (protein C, protein S, and antithrombin)^[79-82]; (4) defects in the fibrinolytic system [low levels of tissue plasminogen activator (t-PA), high levels of plasminogen-activator inhibitor type-1 (PAI-1)]^[83,84]; and (5) elevated number of circulating platelets, platelet activation and increased platelet aggregation tendency^[85-87]. However, in other studies, activation of coagulation was observed both in active and inactive IBD^[88-90], an observation that is in accordance with the occurrence of thromboembolic complications even in IBD patients with quiescent disease.

The hypercoagulable state in IBD has recently been reviewed thoroughly elsewhere^[67]. It can be postulated that, in IBD patients, a persistent latent activation of hemostasis exists in both active and inactive disease states, and is implicated in the thrombotic diathesis and perhaps in disease pathogenesis. Hence, two questions emerge: what is the underlying mechanism for the abnormal hemostasis activation and why do clinically overt thromboembolic complications occur only in a relatively small fraction of IBD patients? A possible explanation for the latter question is the fact that for a thromboembolic event to occur, hypercoagulability alone is not sufficient, and that many other predisposing risk factors have to be present at the same time. On the other hand, for the former question to be answered, one must search deeper into the pathophysiology of intestinal inflammation.

Inflammation and vasculopathy in IBD

As a result of the chronic inflammation in IBD patients, abnormalities exist in both the local intestinal microvasculature and the systemic circulation. Bargen and Barker, almost 80 years ago, stated that, in a subgroup of UC patients, the disease should be described using the term "thrombo-UC"^[3]. Histological studies have revealed vas-

culitis in a subgroup of UC patients^[91], while other studies have shown mucosal capillary thrombi in rectal biopsies from UC and CD patients^[64], although this finding is not specific for IBD^[92]. Wakefield *et al*^[63], proposed that multifocal infarcts in the intestinal microcirculation caused by arteritis with fibrin deposition due to focal vasculitis, might be implicated in CD pathogenesis. Moreover, the proximal demarcation line between involved and uninvolved colon in UC suggests that a microvascular abnormality may be associated with the pathogenesis and the extent of inflammation in UC^[93].

The hemostatic and the inflammatory pathways are closely related in a bi-directional fashion, and the vascular endothelium has been proven to be the interface of their interactions^[68,94-97]. The “vascular hypothesis” suggests that endothelial dysfunction in the intestinal microcirculation plays central role in both UC and CD pathogenesis^[98-100]. Furthermore, the vascular endothelial dysfunction associated with chronic inflammation is critically involved with the hypercoagulable state and the development of thrombosis and atherosclerosis in IBD patients, and clinically manifested as systemic vascular (venous and arterial) thromboembolic complications or IHD^[99-101]. Normally, the “quiescent” intact endothelium exhibits a strong thrombo-resistant surface, expressing antiplatelet, anticoagulant and fibrinolytic properties. An “activated” endothelium is rapidly transformed into a prothrombotic surface, which promotes blood coagulation, inhibits fibrinolysis and activates platelets. The transformation of the vascular endothelial surface from anti-coagulant to pro-coagulant is triggered by mechanical damage, or by perturbation and activation of the vascular endothelial cells. Agents including cytokines, endotoxins, blood mediators, hypoxia, and hemodynamic forces are involved in endothelial cell activation^[102].

Inflammation turns the “quiescent” endothelium into a potent pro-coagulant surface. Interleukin-1 (IL-1), tumor-necrosis factor- α (TNF- α) and other cytokines, which are increased in IBD, are responsible for this pro-coagulant, thrombophilic effect, and increase both white cell and platelet adhesion molecules on the endothelial surface. Many studies suggest that IL-1, TNF- α and other pro-inflammatory cytokines, increase various thrombophilic factors and have a significant contribution in intravascular thrombosis^[103,104].

On the other hand, both thrombosis and “activated” endothelium can promote inflammation. The central role of the endothelial cell in initiation and propagation of inflammation takes place through the recruitment of leukocytes by cell adhesion molecules. The expression of cell adhesion molecules on the endothelial surface is induced by IL-1, TNF- α , and other proinflammatory cytokines. In IBD, the activated endothelial cells express increased surface levels of various intercellular adhesion molecules, such as ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1)^[105]. PECAM-1 (platelet endothelial adhesion molecule-1) is expressed in high levels even in areas of

the colon not affected by UC^[106]. The selectin family (E-, P-, L- selectins), which is involved in leukocyte rolling on the endothelial surface, is increased in IBD^[107]. Furthermore, the CD40/CD40L co-stimulatory pathway, which is involved in inflammation and coagulation, is activated in IBD tissue. In active IBD, CD40 is overexpressed in the microvascular endothelial cells, CD40L is overexpressed in platelets and leukocytes, and the soluble form of CD40L (sCD40L) is increased in the circulation in patients with active disease. The contact of the CD40L+ leukocytes and platelets with the CD40+ endothelial cells in the intestinal microvasculature results in activation of these cells, which in turn promote leukocyte recruitment, platelet aggregation, thrombosis, vascular damage and tissue injury, through a vicious cycle of enhanced production of cytokines and chemokines, and overexpression of adhesion molecules and the tissue factor on endothelial cells^[108-110]. All or some of these molecules are possible therapeutic targets in IBD management^[107-109,111].

Moreover, the production of potent vasoconstrictors from the activated endothelium, such as endothelin-1 and thromboxanes, may contribute to ischemia-reperfusion vascular and tissue injury^[107]. The increased reactive oxygen metabolites (ROMs) found in IBD come from leukocytes and endothelial cells, and may be the products of a recurrent ischemia-reperfusion injury of the vascular epithelium after microthrombi formation^[112,113]. ROMs, in turn, may be implicated in the inflammatory reaction and tissue injury in IBD through activation of NF- κ B factor, which promotes the production of various pro-inflammatory cytokines^[114].

Thrombin, besides its actions in regulating hemostasis, possesses “non-coagulant” functions (Figure 2)^[96,115,116]. Thrombin promotes the production of monocyte chemoattractant protein-1 (MCP-1) from monocytes and interleukins-6 and -8 (IL-6 and IL-8) from fibroblasts, epithelial cells, monocytes and endothelial cells. Thrombin enhances leukocyte adhesion on endothelial cells through induction of endothelial PAF (platelet activating factor) formation^[117-122].

Collectively, a possible pathway in IBD pathogenesis could involve the combination of a “sepsis” model with a persistent, low-grade, controlled and compensated disseminated intravascular coagulation due to infection-induced hemostasis activation^[96,97,123-125] and an “endothelial perturbation-inflammation” model due to ischemia-reperfusion injury^[98,107,126]. It is well known that the increased intestinal permeability in IBD results in an inflammatory reaction in the bowel wall, due to the dysregulated mucosal uptake of luminal bacterial, toxic and antigenic substances. Both the inflammatory reaction and the endotoxemia might promote hemostasis activation and hypercoagulation^[123]. Endotoxin and endotoxin-induced microclots in the systemic circulation have been found in a high proportion of IBD patients^[124,125]. Furthermore, ischemia/reperfusion-induced endothelial dysfunction^[126] promotes inflammation, thrombosis, vas-

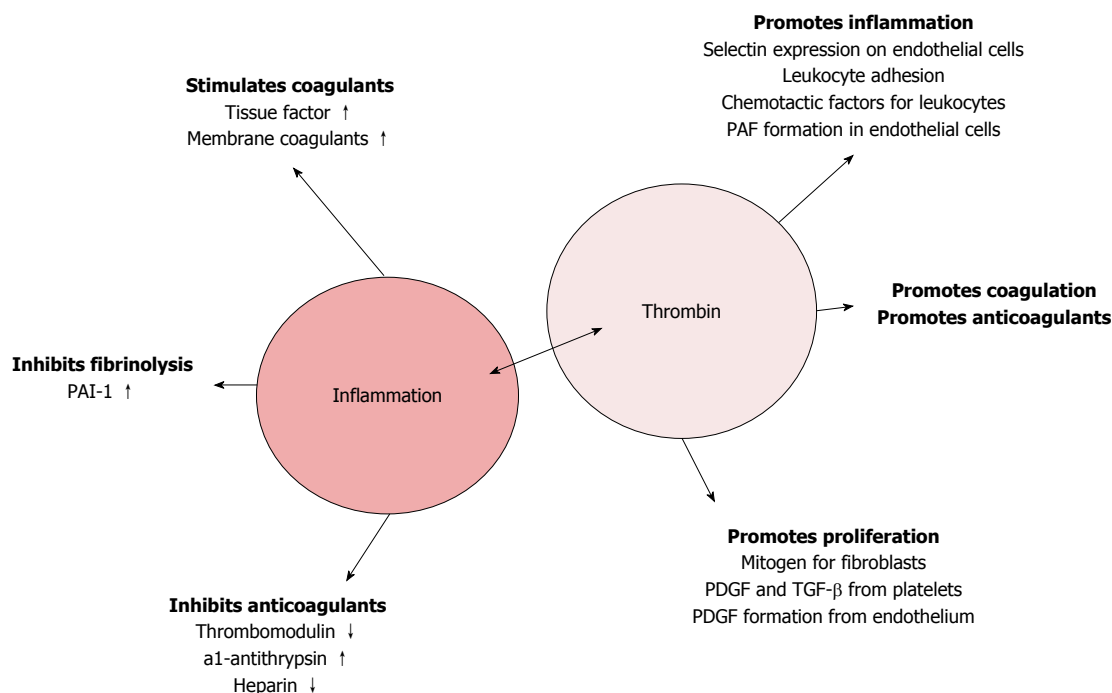


Figure 2 Inflammation and coagulation pathways are interrelated. PAF: Platelet activating factor; PAI-1: Plasminogen-activator inhibitor type-1; TGF: Transforming growth factor.

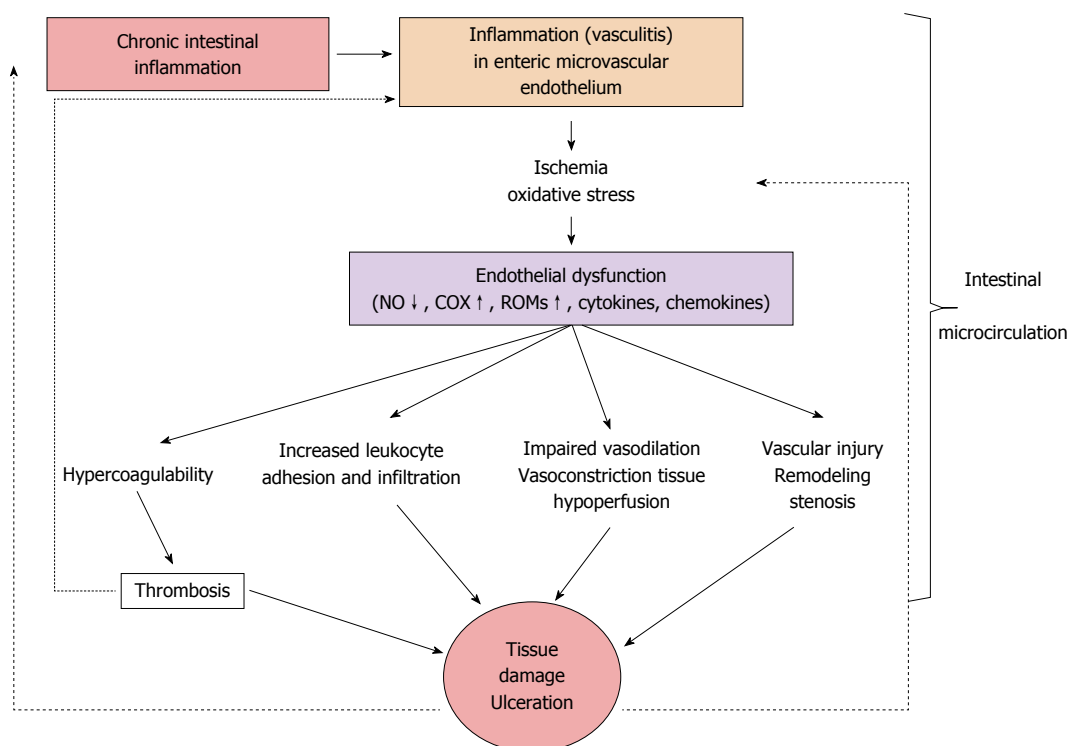


Figure 3 Proposed mechanism of the endothelial dysfunction in the intestinal microcirculation, in inflammatory bowel disease. (Adopted and modified from Hatoum *et al*^[38]). ROMs: Reactive oxygen metabolites; COX: Cyclooxygenase.

Table 5 Management of thromboembolic complications in inflammatory bowel disease patients

Primary prevention of thromboembolic complications	
Ambulatory patients	Hospitalized patients
General measures	General measures
Physician awareness	Disease activity amelioration
Patient education	Early mobilization
Active disease treatment and remission maintenance	Judicious use of catheters
Recognition, elimination or modification of risk factors	Dehydration or nutritional deficiencies restoration
Steroid use	Medication modification
Smoking	Peri-operatively or in severely ill non-surgical patients
Oral contraceptives	Prophylactic anticoagulation (UH or LMWH)
Cardiovascular risk factors and other co-morbidities	Plus mechanical measures when increased thrombosis risk or mechanical measures only, when anticoagulation contraindicated with high bleeding risk
Long-distance flights	
Post-hospitalization period	
Compressive stockings?	
Treatment of a thromboembolic event	
Amelioration of disease activity	
Hematology consultation and thrombophilia screening	
Therapeutic anticoagulation - UH or LMWH	
Thrombolysis - interventional radiology/surgical consultation	
Secondary prevention of thromboembolic complications	
After a first TE episode	
Active disease - spontaneous event	
Short term anticoagulation? - 3 to 6 mo	
Plus anticoagulation during subsequent flares?	
Inactive disease - spontaneous event	
Long term anticoagulation?	
Recurrent TE or inherited thrombophilia	
Hematology consultation	
Long term anticoagulation	

UH: Unfractionated heparin; LMWH: Low molecular weight heparin.

cular anatomic and functional changes, and tissue injury through a self-propagating loop (Figure 3).

MANAGEMENT OF THROMBOEMBOLISM IN IBD PATIENTS

The management of thromboembolism in IBD patients includes primary prophylaxis of first Thromboembolic complication, treatment of a Thromboembolic complication and secondary prophylaxis of the recurrence of a thromboembolic complication (Table 5). Currently, there are no universal and specific guidelines for the management of Thromboembolic in IBD patients in various clinical settings, except for the primary prophylaxis of VTE in hospitalized IBD patients with severe active disease^[127-132].

Primary prevention of venous thromboembolic complications

Hospitalization is an important risk factor for VTEs for many patient groups, including IBD patients^[25,27]. According to the American College of Chest Physicians (ACCP) guidelines for the prevention of VTE^[127],

hospitalized patients with IBD are at a moderate risk (10%-40%) of developing DVT and prophylaxis is the recommended beneficial strategy. The first choice is prophylaxis with a low-molecular-weight heparin (LMWH), low-dose unfractionated heparin (LDUH), or fondaparinux. Mechanical thromboprophylaxis is recommended for patients at high bleeding risk, or if anticoagulants are contraindicated^[127]. International and national IBD organizations and societies in North America and Europe have adopted these recommendations in their recent guidelines for the management of IBD patients requiring hospitalization^[128-132]. The recommendations are more clearly stated for patients with severe UC^[128-131].

However, there are some issues regarding anticoagulation of hospitalized IBD patients which need special consideration. Rectal bleeding is a common symptom in IBD patients (mainly UC) and therefore there is a theoretical concern about worsening the rectal bleeding. Data derived from randomized trials which used UH^[133,134] or LMWH^[135-138] as a treatment for active IBD (UC) and a meta-analysis^[139] showed that although a clear benefit from the heparin use in ameliorating the disease activity was not demonstrated, its use was safe, without major adverse events. Heparin could be an ideal drug for IBD treatment, especially for UC, because of its anticoagulant, anti-inflammatory, immunomodulatory and mucosal healing properties. The failure of the existing trials to prove its efficacy for the UC treatment could be related to the small patient number and the heterogeneity of these studies regarding the compound of LMWH and the dosage administered, the duration of treatment and the definition of response to treatment. Larger studies may be needed to clarify this issue and to reveal the optimal dosing of heparin and the features of a subgroup of patients with active UC who may benefit from LMWH administration.

Recently, Ra *et al*^[140] retrospectively assessed the safety of prophylactic anticoagulation in hospitalized IBD patients. They reported that 80% of the IBD patients received anticoagulation, mainly in the form of LMWH (93%). Anticoagulation administration was more frequent to IBD patients on the surgical service, those with more extensive disease and predominantly those without rectal bleeding. They also found that the rates of major or minor bleeding were not significantly higher in patients who received prophylactic treatment compared to those who did not. The authors concluded that the use of anticoagulation in IBD hospitalized patients is safe, even in the presence rectal bleeding provided that there are no signs of hemodynamic instability^[140].

Another issue which needs discussion is whether anticoagulation prophylaxis should be administered only to severely ill hospitalized IBD patients or to all hospitalized IBD patients. Since hospitalization is an independent risk factor for VTEs in IBD patients and these patients may have additional risk factors for thrombosis (inflammation, catheters, immobilization, complicated disease), it would be reasonable to take measures in order to reduce the

other risk factors by aiming to: ameliorate disease activity, institute early mobilization, use IV catheters judiciously, avoid/treat dehydration and nutritional deficiencies, and minimize medications predisposing to thrombosis. Finally, it would be prudent to expand the indication for prophylaxis to inpatients with IBD who are not necessarily too ill to be confined to bed^[25,27] or even are in remission and hospitalized for other indications, since there is significantly increased risk for VTEs in these groups as well^[25]. Prophylaxis, together with the increased awareness for signs VTEs during the routine clinical assessment of the IBD patients admitted to the hospital, may be more feasible and cost-effective in clinical practice than the use of expensive screening tests^[141].

Another important question that needs an answer is the duration of prophylactic anticoagulation in non-surgical IBD patients after discharge from the hospital. Studies have demonstrated that many VTEs occur during the immediate post-hospital period both in the general population^[142] and in IBD patients^[24,25]. Patients with IBD are discharged from the hospital with improved, but not necessarily fully remitted, disease and to date there are no data for extended VTE prophylaxis during this post-hospitalization period, although its use could be justified in patients with increased risk for thrombosis^[143-145].

Patients with IBD are at increased risk for postoperative VTE^[33,34,43]. According to the data from these studies and to the ACCP guidelines, patients with CD have a moderate risk of VTEs after intestinal surgery, while the patients with UC have a high risk of post-surgical VTEs^[146]. Furthermore, Scarpa *et al*^[147] reported that a standard prophylactic dose of LMWH was inadequate to prevent VTEs in IBD patients with major colorectal surgery and in particular in patients with UC. These data suggest that the perioperative prophylactic anticoagulation in IBD patients should include higher doses of LMWH, for longer periods post-operatively, or even be combined with adjunct mechanical methods^[146-148].

Recent data have confirmed that ambulatory IBD patients with active disease are at increased risk for VTEs^[25,37]. Although, Grainge *et al*^[25] reported that the risk of VTEs was significantly higher during flares and chronic activity periods compared to periods of remission of the disease, both in ambulatory and hospitalized IBD patients, currently there are no guidelines for the primary prevention of VTEs nor is sufficient data for the beneficial use of anticoagulants in the ambulatory setting^[145]. However, general prophylactic measures could also be applied in the ambulatory setting and include: patient education about the risks and the presenting symptoms of thromboembolic complications; enhancement of clinician awareness of this ominous extraintestinal manifestation, with attention to the history and clinical signs of TE in the routine clinical assessment of IBD patients; aggressive treatment of active disease and maintenance of remission; early recognition and elimination or minimization of modifiable risk factors (steroid use, smoking, oral contraceptives, hormone-replacement therapy, long-

distance flights)^[149-151]. Furthermore, in a recent decision analysis study, Nguyen and Sharma^[152] explored the cost-effectiveness of pharmacological VTE prophylaxis in ambulatory IBD patients and concluded that pharmacological VTE prophylaxis in ambulatory IBD patients with acute disease could not be recommended, even though it was beneficial, because it was not cost-effective.

There are no direct data that anticoagulation for VTE prophylaxis in IBD patients actually works since there are no randomized controlled trials that have evaluated this issue yet. However, indirect evidence demonstrated that in acutely ill medical patients pharmacological prophylaxis significantly reduces the incidence of VTE and mortality^[127,143,144].

Primary prevention of ATEs

Apart from the general measures mentioned previously and anticoagulation prophylaxis during hospitalization or post-operatively, clinicians should routinely assess IBD patients for cardiovascular risk (hypertension, diabetes, hyperlipidemia, obesity, hyperhomocysteinemia, positive family history) and preventive measures and/or treatment of these risk factors should be applied^[2].

Therapy of VTE and secondary prevention

The treatment of an acute thromboembolic episode in IBD patients is similar to non-IBD patients. Pharmacological anticoagulation (AC) with UH or LMWH is usually administered in mild to moderate Thromboembolic events, while thrombolysis or catheter-directed thrombolysis (CDT) are reserved for more severe TE including massive thrombosis and organ- or life- threatening vascular occlusion^[2,150]. Therapeutic doses of anticoagulants or thrombolytics for the treatment of TE in IBD patients presents a major safety concern regarding the risk of gastrointestinal (GI) and systemic hemorrhagic complications, since many of the patients have active disease with rectal bleeding. The management decisions should be individualized according to the clinical setting in each patient and episode, and often requires a multidisciplinary approach^[2,150]. The safety of UH and LMWH has been proven in previous studies which evaluated heparin for the treatment of active IBD^[139]. Tabibian *et al*^[153] in a systematic review evaluated the clinical outcomes with anticoagulation and CDT in IBD patients with TE, and reported that both CDT and AC were well tolerated by IBD patients with TE. They suggested that CDT may be used preferentially in patients with severe life-threatening TE, while AC may be more suitable in patients with less clinically significant Thromboembolic or patients at higher risk for bleeding. Furthermore, they demonstrated the safety of these treatments, even when they were used in patients with rectal bleeding, provided that there was no concurrent major GI hemorrhage^[153,154].

The duration of anticoagulation is another important issue because of the increased risk of recurrence of TE in IBD patients. The duration of anticoagulation after initial treatment for Thromboembolic ranges from 3 mo

to lifelong, depending on the individual case. In cases where a Thromboembolic event occurred during active disease, the anticoagulation must be continued at least until clinical remission occurs^[12,154]. In a recent decision analysis study, Nguyen and Bernstein^[155] suggested that lifetime anticoagulation was marginally more beneficial than the time-limited (6-mo) anticoagulation after a first unprovoked VTE in the absence of active disease. They also recommended that in the case of VTE during a flare of the disease, time-limited anticoagulation with or without prophylaxis during subsequent flares would be a more suitable option^[155]. In general, LMWHs, vitamin K antagonists (VKAs; warfarin) or even the new direct oral anticoagulants (NOACs; rivaroxaban, dabigatran, apixaban, and edoxaban) can be used for the long term treatment of TEs. For NOACs new evidence from studies suggests that they have comparable efficacy to that of VKAs with a more favorable safety profile, but there is no direct evidence for their use in IBD patients yet^[156,157].

Physicians' perceptions of the risks and practices in VTE prophylaxis in IBD patients

The risk of thrombosis in IBD patients is high, with significant morbidity and mortality. It is important for the treating physicians to be aware of this serious extraintestinal manifestation and to be able to efficiently recognize and treat the Thromboembolic events. As previously mentioned, international and national IBD organizations and societies in North America and Europe have recently published guidelines for the prevention of TEs in IBD patients^[128-132]. However, surveys which have evaluated the practices of gastroenterologists regarding the issue of VTE prophylaxis in IBD patients have shown that although a significant proportion is aware of the increased risk of TEs in hospitalized IBD patients, their practices for VTE prophylaxis is variable^[158-161].

Razik *et al.*^[158] reported that among 56 Canadian academic gastroenterologists, 55% reported the existence of standard hospital protocols for DVT prophylaxis in hospitalized IBD patients, and more than 80% reported the administration of some form of VTE prophylaxis, but only 50% of them were aware of the existing guidelines. Sam *et al.*^[159] in a similar survey among 135 gastroenterologists in United States, practising mainly in an academic setting (77%), reported that although most of them (84%) had IBD patients with VTE and realized the risks of VTEs, only 67% had protocols for VTE prophylaxis, 45% were aware of the guidelines and finally, 14% would never administer prophylaxis in their IBD inpatients. Gastroenterologists with high volumes of IBD patients were more likely to administer VTE prophylaxis. In addition, Tinsley *et al.*^[160] reported that the awareness of the heparin use for VTE prophylaxis was more frequent among gastroenterologists who were in academic settings, and those who had high volumes of IBD patients, and those who had less than 5 years of practice experience. Finally, Tinsley *et al.*^[161], in another study,

investigated retrospectively the rates of pharmacologic VTE prophylaxis in UC inpatients at a tertiary referral center and concluded that pharmacologic prophylaxis was not ordered or was administered inadequately in a substantial proportion of UC patients admitted in the hospital despite the existing guidelines.

To summarize, all these data clearly show that there are significant variations in practice regarding VTE prophylaxis in hospitalized IBD patients due to a high level of unawareness of current guidelines. It is important for Gastroenterology societies and organizations to more aggressively pursue the education of gastroenterologists, especially those with low volumes of IBD patients, so that they better understand the risks and the adverse outcomes of thromboembolism in IBD patients. The goals are to have them routinely incorporate clinical assessment for signs and symptoms of TEs and to have them efficiently prevent or treat TEs^[162]. Very recently, during finalization of the present review, the Canadian Association of Gastroenterology (CAG) published (in press-on line first) specific recommendations for the prevention and the treatment of VTE in IBD patients in various clinical settings^[163]. The CAG has addressed many of the gaps which exist in the management of VTE in this patient group and has provided a useful and applicable evidence-based guide for the physicians who are involved with the care of IBD patients. The recent CAG guidelines give solid recommendations for some of the important issues we have outlined in Table 5.

CONCLUSION

Evidence from the literature suggests that thrombosis is a specific feature of IBD that can be involved in both the occurrence of thromboembolic events and the pathogenesis of the disease itself. The precise etiology for the higher rates of thromboembolism in IBD and the specific association is as yet unknown, but multiple acquired and inherited factors are implicated. Hypercoagulability is thought to be involved in IBD pathogenesis and future research may reveal potential therapeutic targets for the IBD management. More importantly, both arterial and venous thromboembolic complications are serious and challenging extra-intestinal manifestations to manage, with significant morbidity and mortality in IBD patients. However, thromboembolism is preventable and, therefore, clinician awareness of the risks, and the knowledge of how to efficiently prevent or treat TEs in patients with IBD are of vital importance. Future clinical trials should clarify the ill-defined issues of the thromboprophylaxis in ambulatory patients with active disease, the thromboprophylaxis in patients during the immediate post-hospitalization period, and the duration of thromboprophylaxis. In addition, clinical trials should provide clinicians with reliable methods or markers for assessing the prothrombotic risk in IBD patients in order to promptly apply preventive measures.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Managing osteoporosis in ulcerative colitis: Something new?

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Abstract

The authors revise the latest evidence in the literature regarding managing of osteoporosis in ulcerative colitis (UC), paying particular attention to the latest tendency of the research concerning the management of bone damage in the patient affected by UC. It is wise to assess vitamin D status in ulcerative colitis patients to recognize who is predisposed to low levels of vitamin D, whose deficiency has to be treated with oral or parenteral vitamin D supplementation. An adequate dietary calcium intake or supplementation and physical activity, if possible, should be guaranteed. Osteoporotic risk factors, such as smoking and excessive alcohol intake, must be avoided. Steroid has to be prescribed at the lowest possible dosage and for the shortest possible time. Moreover, conditions favoring falling have to be minimized, like carpets, low illumination, sedatives assumption, vitamin D deficiency. It is advisable to assess the fracture risk in all UC patient

by the fracture assessment risk tool (FRAX[®] tool), that calculates the ten years risk of fracture for the population aged from 40 to 90 years in many countries of the world. A high risk value could indicate the necessity of treatment, whereas a low risk value suggests a follow-up only. An intermediate risk supports the decision to prescribe bone mineral density (BMD) assessment and a subsequent patient reevaluation for treatment. Dual energy X-ray absorptiometry bone densitometry can be used not only for BMD measurement, but also to collect data about bone quality by the means of trabecular bone score and hip structural analysis assessment. These two indices could represent a method of interesting perspectives in evaluating bone status in patients affected by diseases like UC, which may present an impairment of bone quality as well as of bone quantity. In literature there is no strong evidence for instituting pharmacological therapy of bone impairment in UC patients for clinical indications other than those that are also applied to the patients with osteoporosis. Therefore, a reasonable advice is to consider pharmacological treatment for osteoporosis in those UC patients who already present fragility fractures, which bring a high risk of subsequent fractures. Therapy has also to be considered in patients with a high risk of fracture even if it did not yet happen, and particularly when they had long periods of corticosteroid therapy or cumulative high dosages. In patients without fragility fractures or steroid treatment, a medical decision about treatment could be guided by the FRAX tool to determine the intervention threshold. Among drugs for osteoporosis treatment, the bisphosphonates are the most studied ones, with the best and longest evidence of efficacy and safety. Despite this, several questions are still open, such as the duration of treatment, the necessity to discontinue it, the indication of therapy in young patients, particularly in those without previous fractures. Further, it has to be mentioned that a long-term bisphosphonates use in primary osteoporosis has been associated with an increased incidence of dramatic side-effects, even if uncommon, like osteonecrosis of the jaw and atypical sub-trochanteric and

diaphyseal femoral fractures. UC is a long-lasting disease and the majority of patients is relatively young. In this scenario primary prevention of fragility fracture is the best cost-effective strategy. Vitamin D supplementation, adequate calcium intake, suitable physical activity (when possible), removing of risk factors for osteoporosis like smoking, and avoiding falling are the best medical acts.

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Key words: Ulcerative colitis; Osteoporosis; Fragility fracture; Bone mineral density; Trabecular bone score; Hip structural analysis; Fracture assessment risk tool; Dual energy X-ray absorptiometry

Core tip: Diagnosis and treatment of osteoporosis in ulcerative colitis are discussed according to the latest evidence. Innovative applications of new programs derived from bone densitometry to evaluate bone quality and to predict fracture risk in patients affected by ulcerative colitis are described. Charts for ten years fracture risk may be utilized to refer patients to bone densitometry and/or to prescribe drugs against osteoporosis. Trabecular bone score and hip structural analysis may be considered to assess bone quality, that could be impaired by malabsorption and chronic inflammatory status. Advices for prevention and treatment of bone damage are given, also considering cost-effectiveness.

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INTRODUCTION

Osteoporosis is a well known extraintestinal manifestation of the inflammatory bowel diseases (IBD), more common in Crohn's disease (CD) than in ulcerative colitis (UC). Nowadays its management has been recognized as a relevant aspect in patients' follow up. As IBD is a chronic condition lasting the whole life of the patient, the effects of the disease and of its treatment, together with the ageing of the population, determine an increase in the prevalence and in the incidence of osteoporosis. The present article revises the latest evidences (2009-2014) in the literature regarding this theme, paying particular attention to the latest tendency of the research concerning the management of bone damage in the patient affected by UC. The authors have consulted PubMed, Embase, Cochrane Library, National Institute for Health and clinical excellence. In addition, the official positions of the gastroenterological societies and the leading guidelines for the management of osteoporosis have been examined.

ULCERATIVE COLITIS

UC is a chronic inflammatory disease of colon and rectum. The disease reaches from the anal verge to a variable proximal extension of the colon. The main peak incidence is between the second and the fourth decade, but the disease may also start later. Both men and women are affected with a similar frequency.

Aetiology is unknown. Regarding pathogenesis, the inflammation is probably caused by a pathologic immune response to an unknown environmental stimulus in the lumen of the colon in genetic susceptible people. A defective colonic epithelium may allow commensal bacteria to be sampled by dendritic cells of the mucosa and act as antigenic stimulus to induce an immune response, sustained by T-cells, leading to inflammation^[1].

Symptoms are rectal blood and mucus, tenesmus, urgency and diarrhoea, depending on the extent and the severity of the disease. Usually UC is classified in mild, moderate and severe, depending on the clinical manifestations according to the Montreal criteria^[2] (Table 1).

About one third of patients presents immune mediated inflammatory extra intestinal manifestations, which may affect liver and biliary system, joints and bone, skin, eyes.

The usual course of UC presents periods of acute inflammation and phases of remission of symptoms. In rare cases there is only one flare of disease, which can be very severe ("fulminant" colitis). The recurrences are variable in extent and severity and unpredictable in timing. Aim of the therapy is to induce the remission and to maintain it as long as possible.

Systemic and/or topical therapy is focused against pathologic immune response. Mainsteps of the pharmacological treatment are 5-aminosalicylic acid, glucocorticoids, azathioprine and its derivative 6-mercaptopurine, cyclosporine and biological agents such as infliximab and adalimumab.

Bone implication of UC is represented by osteoporosis, even if it is less frequent than in CD.

OSTEOPOROSIS

Osteoporosis is defined as a systemic disease characterized by low bone mass and micro-architectural deterioration of the bone tissue, with a consequent increase in bone fragility^[3]. The compromised bone strength leads to an increased risk of fracture, as bone strength reflects the integration of bone mineral density (BMD) and bone quality^[4]. The disease typically occurs in postmenopausal women and in the elderly people (primary osteoporosis) or in patients with diseases affecting bone mineral metabolism, like IBD (secondary osteoporosis). Also glucocorticoid treatment is a well-known factor leading to osteoporosis.

Common sites of osteoporotic fractures are the spine, the hip, the distal forearm and the proximal humerus. Osteoporotic fractures also occur at many other sites in-

Table 1 Montreal classification of extent and severity of ulcerative colitis

Extent	Anatomy	Severity	Definition
E1: Ulcerative proctitis	Limited to the rectum	S0: Clinical remission	Asymptomatic
E2: Left sided (distal) ulcerative colitis	Limited to a proportion of the colorectum distal to the splenic flexure	S1: Mild	≤ 4 stools/d (with or without blood), absence of systemic illness, and normal inflammatory markers
E3: Extensive (pancolitis) ulcerative colitis	Extends proximally to the splenic flexure	S2: Moderate S3: Severe	> 4 stools/d but minimal signs of systemic toxicity ≥ 6 bloody stools/day, pulse ≥ 90 beats/min, temperature ≥ 37.5 °C, haemoglobin < 1.05 g/L, and erythrocyte sedimentation rate ≥ 30 mm in the first hour

Adapted from: Ford *et al*^[1].

Table 2 Effect on vertebral fracture rates (from randomized controlled trials)

	Osteopenia	Osteoporosis	Established
Raloxifene	•	■	■
Alendronate	NA	■	■
Risedronate	NA	•	■
Ibandronate	NA	■	■
Zoledronate	NA	■	■
Teriparatide	NA	NA	■
Strontium ranelate	•	■	■
Denosumab	NA	■	■

■ Denotes a preplanned analysis in the entire study population; • Denotes a post hoc analysis. NA: No evidence available. Adapted from: Body *et al*^[12].

cluding pelvis, ribs and distal femur and tibia.

Osteoporotic fractures are associated with high rates of disability and mortality. Approximately 50% of fracture-related deaths in women are due to hip fractures, 28% to clinical vertebral fractures and 22% to other fractures^[3]. In individuals who experience hip fractures, 20% die within the next year and 20% will require permanent nursing home care^[4-6]. Vertebral fragility fractures are the most frequent fractures in osteoporotic patients, and even if this kind of injuries has less severe complications than hip fractures, they are associated with substantial disability due to impairment in spine dynamics and static biomechanics. Furthermore, the number and the severity of vertebral fractures are related to an exponential increase of subsequent fractures^[7,8].

The gold standard method for the diagnosis of osteoporosis is the dual energy X-ray absorptiometry (DXA) which is a radiological tool based on the principle of photon absorptiometry developed in the sixties^[9] that allows to quantify the BMD^[10].

Bone densitometry scans for diagnostic classification and prediction of fracture risk are performed according to the international guidelines^[11] on lumbar spine and proximal femur, which are the most important sites of fragility fractures. Absolute values of BMD are expressed as T-score and Z-score. T-score is calculated as standard deviation from the normal reference population, and Z-score is calculated as standard deviation from the sex and age matched population. Osteopenia is defined as a T-score between -1 and -2.5. Osteoporosis is defined as a

T-score < -2.5^[10]. Low BMD is directly correlated with an increase in fracture risk^[3].

The goal of the pharmacological therapy of osteopenia and osteoporosis is to increase bone strength, in order to decrease the risk of fracture^[3] mainly by increasing BMD. This can be achieved by decreasing bone resorption and/or increasing bone formation.

A lot of effective medications are approved for the prevention and the treatment of osteoporosis. Drugs can be divided into two categories: anti-resorptive (or anti-catabolic) and anabolic agents. Anti-resorptive agents, which include oestrogens, selective oestrogen receptor modulators (raloxifene), calcitonin, bisphosphonates (alendronate, risedronate, ibandronate, zoledronate) and denosumab, reduce osteoclast activity, preserving bone mineral density. The currently used anabolic agent is teriparatide (PTH 1-34) which stimulates osteoblast activity. Strontium ranelate is another agent that reduces fracture risk. It improves bone strength mainly through effects on bone formation and bone properties^[3], even if it also has an anti-resorptive action.

The anti-fracture efficacy of the most frequent used drugs for osteoporosis is illustrated in Tables 2 and 3.

The effectiveness of drugs in primary and secondary prevention of fragility fracture is quite different. Regarding vertebral fractures, all the considered drugs are effective in preventing secondary osteoporotic fractures, whereas not all these drugs are also effective in primary prevention. No effectiveness is found in reduction of vertebral fracture rate in osteopenic patients. There are very few evidences regarding the prevention of hip and non-vertebral fractures: only few drugs seem to be useful, and among them only some of the bisphosphonates and denosumab^[3,12].

In addition, to enhance the effectiveness of the pharmacological treatment, calcium and vitamin D supplements may be prescribed. In fact, vitamin D deficient patients do not show the same increase in bone mass and reduction in fracture rate as observed in vitamin D repleted patients^[13].

Moreover, in order to improve bone mass, some changes in lifestyle are suggested, such as increasing physical activity, stop smoking, avoiding excessive alcoholic intake, maintaining an adequate body weight. Furthermore, in order to reduce fracture risk also the prevention of falling is important; so it is advisable to avoid inappropriate housing conditions (*e.g.*, carpets, low illumination), and the use

Table 3 Effect on nonvertebral/hip fracture rates (from randomized controlled trials)

	Nonvertebral		Hip	
	Osteoporosis (without prevalent vertebral fractures)	Established osteoporosis (with prevalent vertebral fractures)	Osteoporosis (without prevalent vertebral fractures)	Established osteoporosis (with prevalent vertebral fractures)
Raloxifene	NA	●	NA	NA
Alendronate	■	■	NA	■
Risedronate	NA	■	NA	■
Ibandronate	NA	●	NA	NA
Zoledronate	■	NA	■	NA
Teriparatide	NA	■	NA	NA
Strontium Ranelate	●	■	●	▲
Denosumab	■	NA	■	NA

■ Denotes a preplanned analysis in the entire study population; ▲ Denotes a preplanned analysis on a subset of the study population; ● Denotes a post hoc analysis. NA: No evidence available. Adapted from: Body *et al*^[12].

of hypnotic and sedative drugs^[12].

Patients should also be aware of the importance of the adherence to the treatment. In fact, it has been observed that only a low percentage (about 30%) of patients still follows therapy after one year^[14].

ULCERATIVE COLITIS AND OSTEOPOROSIS

In literature it has been estimated that osteopenia in UC is found in about 35% of the patients, and osteoporosis in about 15%, based on DXA scans^[15]. However, studies about the prevalence of osteopenic/osteoporotic fractures in patients with UC are scarce, with a small sample size and with a follow up that is not sufficiently prolonged to allow fracture detection^[16]. Another bias is that investigated IBD patients' groups are not homogeneous regarding age, gender, severity and activity of disease, and type of treatment (steroids, supplementation of calcium and vitamin D). A recent retrospective ten years database analysis on UC male patients found a prevalence of fragility fractures of 7.9% in osteoporotic patients, 4.4% in osteopenic patients and 1.1% in patients with normal BMD^[17]. Low bone mass in UC is also related to the severity of the disease, in so far mild and moderate UC seem not to represent a risk factor for osteoporosis^[18]. Obviously, severe UC presents a higher level of inflammation and therefore the need of steroid administration, which are per se risk factors for bone loss and fracture.

Many factors have been suggested to be implicated in the pathogenesis of osteoporosis in UC. They are mainly classified in two groups: nutritional factors and inflammatory mechanisms.

In UC patients poor nutrition and malabsorption, particularly of calcium, vitamin D and K, can be caused by anorexia, insufficient diet, diarrhoea. On the other hand, IBD associated inflammatory cytokines [interleukin 1 (IL1) and 6 (IL6), tumour necrosis factor alfa (TNF- α)] have been shown to affect bone metabolism directly^[19]. These cytokines are known to increase synthesis of receptor-activated nuclear factor K B ligand (RANKL), which is produced by osteoblasts and which activates proliferation

and differentiation of osteoclasts, thereby inducing bone resorption^[19]. In fact, the biological drugs inhibiting TNF alfa have been shown to increase BMD^[19]. Moreover, recent studies in animals have indicated that these cytokines also negatively act on intestinal and renal absorption of minerals and vitamins^[20]. Of course, the usual risk factors for osteoporosis may also be present in UC patients, such as low body mass index, older age, immobilization, smoking, prolonged use of corticosteroids, low peak of bone mass particularly in the case of paediatric onset of the disease.

Anyway, the two most important factors for developing OP in UC seem to be systemic inflammatory activity and the prolonged use of glucocorticoid. Corticosteroid treatment in UC patients is related to the inflammatory status, being cumulative doses of steroid directly related both to the severity of UC and the risk of low BMD^[17].

Glucocorticoids act negatively on bone mineral metabolism inducing an impairment of bone cells coupling and activity, stimulating osteoclasts formation and activity, promoting osteoblasts apoptosis, inhibiting osteoblasts proliferation and synthesis of type I collagen and osteocalcin. In addition, they reduce intestinal absorption of calcium and increase urinary calcium excretion, leading to an increase in PTH secretion. Moreover, they reduce sex hormones production by inhibiting hypothalamic-pituitary-gonadal axis^[21].

It has to be kept in mind that all these actions of glucocorticoid finally lead to a significant increase in bone loss and in vertebral and non-vertebral fracture risk. This effect is already observed in the first three-six months of steroid treatment, and it is already present with daily doses of 5 or more milligrams of prednisone equivalent^[22].

SOMETHING NEW IN THE MANAGEMENT OF OSTEOPOROSIS IN ULCERATIVE COLITIS

The most cited scientific gastroenterological societies such as American Gastroenterological Association^[23], American College of Gastroenterology^[24], British Society of Gastroenterology^[25], have dealt in their guidelines with

the management of osteoporosis in IBD patients. They give general recommendations regarding prevention and removing of fracture risk, indication of DXA test and prescription of specific pharmacological treatment of osteoporosis. These suggestions do not substantially differ from guidelines for prevention, diagnosis and treatment of primary and secondary osteoporosis in the general population^[26-31].

In recent years, besides DXA test, another tool for assessing fracture risk has been proposed by the Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield (United Kingdom), and introduced in clinical practice.

Prediction of fracture risk

Fracture risk assessment tool (FRAX[®] tool - <http://www.shef.ac.uk/FRAX/>^[32]) is based on the collection of the main anagraphic, anthropometric and anamnestic data regarding fracture risk factors, with or without BMD. This collection allows to compile a chart, available both for men and women aged 40 years or more, that predicts the 10-year probability of a hip fracture or of a major osteoporotic fracture, such as clinical spine, hip, forearm and humerus fractures. At the moment these charts are prepared for 59 countries worldwide.

Frax score is calculated by online compiling twelve fields of an algorithm: age; gender; height and weight; history of minimal trauma fractures; history of parental hip fractures; corticosteroid exposure (defined as oral glucocorticoids for more than 3 mo at a dose of prednisolone equivalent of 5 mg daily or more); concomitant rheumatoid arthritis; secondary strongly associated causes of osteoporosis, such as type I (insulin dependent) diabetes, osteogenesis imperfecta in adults, untreated longstanding hyperthyroidism, hypogonadism or premature menopause (< 45 years), chronic malnutrition, malabsorption and chronic liver disease; more than three units of daily alcohol intake; smoking. Lastly, if available, BMD may be inserted, expressed as absolute value (g/cm^2) or T-score.

Entering these variables one can obtain a number, that quantifies the probability of having a major or a hip fracture in the subsequent ten years. This parameter helps the clinician in the decision whether to prescribe or not a pharmacological treatment for osteoporosis, according to a threshold of intervention. This threshold is not uniquely established and has not the same cut-off for all countries, depending on the clinical contest and health economic factors. The fracture risk varies markedly between the different countries, whereas the T-score varies only by a small amount. In addition, the clinical interpretation of a given T-score for fracture risk in women of every country depends on the age and on the presence of clinical risk factors. Intervention thresholds are also partly determined by the willingness to pay for health care in osteoporosis and by the access to DXA, which vary from country to country^[29].

The use of FRAX tool in assessing fracture risk in

UC patients was examined only in two retrospective studies^[15,33]. Results are controversial, but encourage to explore the utility of this clinical tool in the management of IBD. The first^[15] shows that the clinical FRAX score alone can accurately predict the risk of a osteoporotic fracture, reducing the need for DXA scans and for unnecessary pharmacological treatment for osteoporosis. As indicated in a position paper of the National Osteoporosis Guideline Group (NOGG)^[34], if the patient results at low risk, no DXA scan is required. If the FRAX score, based only on clinical risk factors, is intermediate, patients should undergo DXA scanning and once the BMD is known, the FRAX score has to be recalculated to determine the need for specific anti-osteoporosis treatment.

The Authors of the first cited study^[15] confirmed an increased prevalence of osteoporosis in their UC population and found that by using the FRAX score over 5 years they could have avoided 36% of DXA scans. They also found that patients who carry a high clinical risk of fracture are frequently not considered for treatment when this decision is based on T-score alone. Furthermore, considering NOGG guidelines^[35], 8% of the patients examined in the study were over-treated with bisphosphonates. As illustrated by the Authors there are, however, some limitations in using FRAX tool in IBD patients. First, the FRAX algorithms are based on general population cohort studies undertaken in over 40 years old people, and have not been validated in IBD populations, where younger, under 40 years old people are frequently represented. In this condition FRAX tool calculates the risk for patients under the age of 40 using the data of individuals aged 40 years or more. It is likely, therefore, that the fracture risk in IBD subjects could be overestimated. It will be useful to collect specific data for patients under 40, particularly in IBD populations, but probably it will take several decades to complete a prospective cohort observation in young patients for the collection of an adequate number of fractures. Second, body mass index, a component of the FRAX score, in the general population does not fluctuate at the same rate as in IBD, where periods of active disease may cause substantial weight loss. Third, although the FRAX score includes corticosteroids use as a dichotomous risk factor (yes/no), it does not take into account the cumulative dose of steroids.

The other cited retrospective study^[33] in IBD patients, raising from a population database of the province of Manitoba (Canada), examined the risk of major osteoporotic fractures (MOF) and of hip fractures after controlling for FRAX, independently from FRAX probability. The Authors did not find an increase in the risk of MOF in IBD patients after having controlled the FRAX probability, estimated both with and without BMD. The results for patients with CD and UC considered separately were similar. They found, conversely, an increase in the risk of developing a hip fracture, even after having controlled the FRAX probability, estimated both with and without

BMD. Due to the small number of events (hip fracture) it was not possible to compare UC and CD. The addition of the femoral neck BMD to calculate the risk did not significantly increase the estimated risk for hip fractures associated with IBD, suggesting, therefore, that IBD exerts a BMD independent effect on hip fracture risk. It may be useful that further studies confirm these results, differentiating UC from CD. It would be also desirable that IBD would be added as anamnestic dichotomous factor in FRAX risk calculation.

Assessment of bone structure

DXA represents the gold standard method for the diagnosis of osteoporosis^[10]. However, while BMD is clearly one of the major determinants of bone strength^[36], the assessment of fracture risk by BMD could lack sensitivity. In fact, many fragility fractures occur in osteopenic individuals (T-score between -2.5 and -1.0), not only in subjects with osteoporosis (T-score < -2.5)^[37]. Other factors in addition to BMD account for bone strength and fracture risk, like bone geometry and bone microarchitecture, that concur to determine bone quality^[38].

The best method for the direct assessment of bone micro-architecture is histomorphometry of the transiliac crest bone biopsy, but it is an invasive procedure and, moreover, it does not necessarily reflect microstructure at sites where the fragility fractures occur, like spine and femur. A number of techniques have been developed to assess bone geometry, as quantitative computed tomography (QCT)^[39,40], high resolution peripheral quantitative computed tomography (HRpQCT)^[41] and magnetic resonance imaging^[42]. However, these techniques present more invasivity, higher costs, long time for their execution (long lasting scans). Alternatively, adaptation of X-ray based images, like plain radiographs, using gray-level textural features, have been tested, utilizing fractal dimension^[43-45] and Fourier analysis^[46-48]. An ideal solution, in terms of practicability, costs and risks, could be the adaptation of DXA-based images. DXA can be used to identify existing vertebral fractures^[49-52], to evaluate hip geometry and to estimate femoral strength^[53-55]. Moreover, a new DXA-based technique that considers bone mineral distribution in the proximal femur, instead of only bone mineral density, may be well-suited to enhance standard densitometric evaluation as a predictor of hip fracture risk^[56]. The latest development is the trabecular bone score (TBS), a new gray-level textural measure that can be extracted from the 2-dimensional lumbar spine DXA image to estimate trabecular microstructure. TBS may provide skeletal informations that are not captured by the standard BMD measurement. Based on experimental variograms of the projected DXA image, TBS has the potential to discern differences in 3-dimensional (3D) micro-architecture between 2-D DXA measurements that are similar to each other^[57,58]. An elevated TBS value correlates with better skeletal texture (a reflection of better microarchitecture); a low TBS value correlates with weaker skeletal texture (a reflection of degraded

microarchitecture). The relationship between TBS texture parameters and 3D micro-architecture parameters has been documented by several ex vivo studies that have reported significant correlations between TBS and various micro-structural parameters of bone assessed by micro-computed tomography^[57,59,60].

TBS is an imaging technique adapted directly from the DXA image of the lumbar spine. Thus, it is potentially readily and widely available. In recent years, a large number of studies have demonstrated that TBS is significantly associated with direct measurements of bone microarchitecture, and may be a useful adjunct to BMD for detection and prediction of fragility fractures in primary osteoporosis^[58]. Thus, it promises potential utility also in secondary causes of osteoporosis^[61]. In some conditions, like glucocorticoid-induced osteoporosis and in diabetes mellitus, the TBS appears to out-perform DXA. It also appears useful in numerous other diseases associated with diminished bone health, such as primary hyperparathyroidism, androgen-deficiency, hormone-receptor positive breast cancer treatment, chronic kidney disease, and autoimmune diseases like rheumatoid arthritis^[61]. Further research is required to establish clearly the role of TBS in these and other disorders that adversely affect bone health, like CD and UC.

In adjunction to BMD measurement, TBS can be a useful tool also for monitoring treatment efficacy over time.

Assessment of hip geometry

Hip geometry, like BMD, has been shown to relate independently to hip fracture risk^[62]. Loading forces on bone are distributed over the bone material in cross sections. The concentrations of loading forces, defined stresses, are a function of bending moments and cross sectional geometry. Based on the principle described by Martin and Burr^[53,63] a specific program for bone densitometry has been developed, named hip structural analysis (HSA), that derives the cross sectional geometry from images acquired from a bone mineral scanner by the means of DXA. The main structural parameters are the surface area of the bone in the cross section (CSA) and the section modulus (Z), which are inversely related to maximum stresses due to axial and bending loads, respectively^[64,65]. CSA is an index of bone resistance to axially directed loads. Z is computed from the cross sectional moment of inertia (CSMI) that weights the area in the cross section by the square of its distance from the centroid. CSMI reflects the flexural strength and is an index of structural rigidity. The maximum distance between the center of mass and outer cortex over the average cortical thickness provides a stability index of the cortex under compressive loads, bending included, the so called buckling ratio.

A few works have been published about HSA in primary and secondary osteoporosis^[62,64,66-68] and to estimate bone quality variations after pharmacological treatment for osteoporosis^[69]. A combination of BMD assessment

and geometric structural measurements may represent an additional and helpful mean in estimating bone strength and fracture risk. After therapy, particularly with new bone formation agents, changes in axial and bending strength and, for some drugs, in cortical thickness, are expected.

No data about these topics are available in UC patients and their investigation could be of considerable interest.

Treatment of osteoporosis in ulcerative colitis

Calcium and vitamin D represent a well-known non-pharmacological treatment for osteoporosis, which is usually employed in conjunction with drugs for primary and secondary fragility fracture prevention. Measurement of serum 25-OH-cholecalciferol is the standard method to assess vitamin D status. A recent point of view of the Institute of Medicine of the United States National Academies^[70], considers at least 50 nmol/L (20 ng/mL) the sufficient level for the general healthy population and suggests a daily global intake of vitamin D of about 700-800 IU for the general population. However, this position is not overall accepted and other recommendations suggest higher doses of vitamin D intake for healthy adults^[71].

Vitamin D insufficiency is a condition associated with rickets and osteomalacia, reduced muscle strength, reduced appendicular muscle mass, increase in muscle pain, increase in body sway and consequent risk of falls, and reduced response to osteoporosis treatment. For insufficiency [30-50 nmol/L (12-20 ng/mL)] or deficiency [< 30 nmol/L (12 ng/mL)] a greater amount of vitamin D supplementation is required, identified by IOM up to a maximum daily dose of 4.000 IU. In this case, a periodic check of the vitamin D status is necessary (every 1-3 mo), considering the extreme individual variability in the response, due to various clinical conditions such as malabsorption.

In UC vitamin D insufficiency and deficiency are common, ranging from 45% to 60% of patients^[72,73]. Therefore, it appears advisable to routinely check 25OH-vitamin D in UC patients in order to identify and treat adequately the vitamin D pathological status. Vitamin D in association with calcium has been used in the treatment of osteoporosis in UC, without significant BMD improvement.

In the last decade vitamin D has gained a new surge of scientific interest for its extra-skeletal effects beyond its action on bone metabolism. The vitamin D receptor (VDR) has been isolated in tissues other than intestinal epithelium, distal renal tubules and osteocytes: adrenals, parathyroids, pituitary gland, mammary gland, ovary, testis, skin, heart, thymus, lymphocytes and promiocytes, hepatocytes, biliary epithelial cells and colon. Also 1 α -hydroxylase, the vitamin D activating enzyme, is expressed in colonic cells^[74,75]. A local production of 1,25(OH) $_2$ -vitamin D has been found in skin, lymph nodes, pancreas, brain, adrenal medulla, monocytes

and macrophages, and colon^[76]. A recent meta-analysis showed that higher blood 25OH vitamin D levels were associated with a reduced risk of colorectal cancer: pooled adjusted OR was 0.94 per 10 nmol/L increase in 25OH-vitamin D concentration^[77]. All these experimental data indicate that vitamin D plays a role in the function of the cited tissues and organs. It is notable that in colonic biopsies' specimens of patients with UC both VDR expression and VDR protein are reduced respect to normal. Therefore it is hypothesised that vitamin D supplementation may be useful in UC patients not only for osteoporosis treatment purpose, but also for its extra-skeletal actions.

Moreover, in UC patients various other actions of vitamin D might be useful, considering its involvement in inflammation and immune modulation: reduction of inflammatory cytokines^[78,79], protective immune modulating properties^[80,81], maintenance of integrity of the epithelial barrier of the colon^[82].

Few original clinical trials regarding medical treatments for low bone mass in UC have been published in literature since the eighties. A recent systematic review and meta-analysis^[83] has listed only nine works concerning anti-osteoporotic drugs in UC patients. However, populations considered in these studies are mainly not homogenous, including both CD and UC, male and female, pre- and post-menopausal women, active and non-active disease, different steroid exposure. Within the analyzed anti-osteoporotic treatments, there are variable concomitant medications, with different dosages of calcium and vitamin D supplementation. Seven of the cited nine studies have used anti-osteoporotic drugs and two non-pharmacological treatments (calcium and vitamin D supplementation). Among the seven using drugs, six utilized anti-resorptive agents (bisphosphonates and calcitonin) and one an agent stimulating bone formation (fluoride). Also the duration of the studies is quite different, ranging from few weeks to few years; this is important because the effect of osteoporosis treatment on bone mass and fracture risk is assessable in a reliable way only on a long period, lasting several years. Moreover, not all these studies are of outstanding quality.

For all these reasons it is difficult to draw firm conclusions, and consequently, definitive recommendations.

To our knowledge in the last five years only three clinical trials dealt with anti-osteoporotic drugs in UC^[84-86]. These Authors have utilized bisphosphonates (alendronate, risedronate) and calcitonin. Kitazaki and Kriel used bisphosphonates to prevent glucocorticoid associated osteoporosis in active UC disease. Alendronate improved spine BMD after one year of treatment in a steroid treated UC population very heterogeneous for age (17-70 years, mean age 41 years)^[84]. Kriel administered risedronate for a very short period (two months)^[85]. Pappa prescribed calcitonin to study its short term efficacy on spine BMD in UC children and adolescents, without seeing significant clinical advance^[86].

Amino-bisphosphonates, like alendronate and risedro-

nate, inhibit the enzyme farnesylpyrophosphate-synthase implicated in the biosynthesis of cholesterol. This action significantly reduces the prenylation of GTPase proteins, thus disrupting function of osteoclasts, leading to their apoptosis^[87]. Experimental data in cell culture have shown that the described bisphosphonate action occurs also in various cancer cell lines^[88]. GTPase proteins have been found to be involved in cancer of colon and rectum^[89,90]. Being very poorly absorbed (less than 1%), oral bisphosphonates reach the colon and come in contact with the intestinal epithelium. Thus, they could lead colon cancer cells to apoptosis with the same mechanism^[88]. Moreover, there are experimental evidences that bisphosphonates could hinder the growth of colon and rectum cancer inhibiting macrophages^[91,92] and stimulating a subset of T-cells^[93,94] involved in cancer developing. This potential therapeutic effect could be relevant in UC, where the risk of developing a colorectal cancer is notoriously increased.

FINAL CONSIDERATIONS ABOUT MANAGING OSTEOPOROSIS IN ULCERATIVE COLITIS

Osteoporosis in UC patients is a high prevalent and a high incident pathology, and fracture prevention is a mandatory question. On the other hand, there are scarce evidences about this issue, and therefore it appears not reasonable to give specific, population-based-approach recommendations about primary and secondary prevention of fragility fractures in UC patients. So it may be more advisable to suggest an individual-high-risk-approach, inspired by the consolidated guidelines for the diagnosis and treatment of post-menopausal osteoporosis.

First step

It is wise to assess vitamin D status in UC patients to recognize who is predisposed to low levels of vitamin D. Serum levels of 25OH-vitamin D could be measured in all patients and particularly in those who present well-known risk factors for deficiency: severe disease, elderly patients, reduced sun light exposure. Deficiency has to be treated, preferably with an oral daily cholecalciferol or calcifediol supplementation. Intermittent large doses, orally or parenterally, should be reserved in the case of reduced adherence to therapy. An adequate dietary calcium intake or supplementation and physical activity, if possible, should be guaranteed.

Osteoporotic risk factors such as smoking and excessive alcohol intake must be avoided. Steroid has to be prescribed at the lowest possible dosage and for the shortest possible time. Moreover, conditions favouring falling have to been minimized, like carpets, low illumination, sedatives assumption, vitamin D deficiency.

Secondly

It is advisable to predict the ten years fracture risk in all

UC patient by the FRAX[®] tool, that calculates the risk for many countries of the world for the population aged from 40 to 90 years.

The use of FRAX in clinical practice demands a consideration about the fracture probability at which it is useful to intervene, both for treatment (intervention threshold) and for BMD testing (assessment threshold).

Assessing fracture probability could be useful to help physicians in deciding whether to treat or not for osteoporosis in order to prevent fragility fractures. A high risk value could indicate the necessity of treatment, whereas a low risk value suggests a follow-up only. An intermediate risk supports the decision to prescribe BMD assessment and a subsequent patient revaluation for treatment. The thresholds are variable, since they depend critically on local factors varying from country to country, like fracture incidence, willingness and capability to pay for access to BMD measurement and for health care in osteoporosis. Different scenarios are represented for example by The National Osteoporosis Foundation recommendation for the United States (www.nof.org) and by The National Osteoporosis Guideline Group (NOGG) for the UK (www.shef.ac.uk/NOGG/)^[35].

Thirdly

Bone densitometry could be used not only for BMD measurement, but also to collect data about bone quality by the means of TBS and HSA assessment. These two indices could represent a method of interesting perspectives in evaluating bone status in patients affected by diseases like UC, in which there may be an impairment of bone quality as well as of bone quantity. Bone quantity accounts for most, but not for all, of the fragility fractures. No data are published about TBS and HSA in UC population, and this could be an interesting field for research.

Fourthly

In literature there is no strong evidence for instituting a pharmacological therapy in UC patients for clinical indications other than those that are applied to the patients with established osteoporosis.

Therefore, a reasonable advice is to prescribe pharmacological treatment for OP in those UC patients who present fragility fractures, that bring a high risk for subsequent fractures. Therapy has also to be considered in presence of a high risk of fracture, particularly when corticosteroid therapy is prolonged and with high cumulative doses. In patients without fragility fractures or steroid treatment, fracture risk assessment could support the medical decision about treatment, and in this case FRAX could be of relevant help.

Among drugs for osteoporosis the bisphosphonates are the most studied, with the best and longest evidence of efficacy and safety. Despite this, several questions are still open, such as the lasting of treatment, the necessity to discontinue it, the indication of therapy in young patients, particularly in those without previous fracture.

Further, a long-term bisphosphonates use in primary osteoporosis has been associated with an increased incidence of dramatic, even if uncommon, side effects, like osteonecrosis of the jaw and atypical sub-trochanteric and diaphyseal femoral fractures.

UC is a long-lasting disease and the majority of patients are relatively young. In this condition primary prevention of fragility fracture is the best cost-effective strategy. Vitamin D supplementation, adequate calcium intake, suitable physical activity (when possible), removing usual risk factors for osteoporosis (like smoking), and avoiding falling, are the best and the cheapest medical acts.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Advances in treatment of ulcerative colitis with herbs: From bench to bedside

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their involvement in UC treatment are discussed.

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Key words: Inflammatory bowel disease; Herbs; Herb medicine; Ulcerative colitis; Therapy; Safety

Core tip: Herbal medicine has already been used for some diseases including infections and headache in China since the third century BC. Recently, herbs have emerged as a useful treatment for ulcerative colitis as shown by clinical trials. A better understanding of the herbal bioactivities may provide new alternatives to our current treatment for ulcerative colitis.

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Abstract

Ulcerative colitis (UC), an idiopathic inflammatory disorder in the colon, has become a clinical challenge, owing to the increasing incidence and poor prognosis. The conventional treatments for UC including aminosalicylates, corticosteroids, and immunosuppressants, induce remission in only half of patients. Meanwhile, the treatments often come with serious side effects which can be life-threatening. Herbal medicine, one of the most common traditional Chinese medicine modalities, has been introduced for centuries into clinical treatment of many human diseases such as infections and functional disorders. Recently, the potential effectiveness of herbs has been suggested as the treatment of UC, as shown by a variety of clinical trials and experimental studies. The herbs reported in the literature include *aloe vera* gel, butyrate, tormentil extracts, wheat grass juice, and curcumin. In the review, bioactivity of the herbs and

INTRODUCTION

Ulcerative colitis (UC), one type of inflammatory bowel disease (IBD), is characterized by uncontrolled inflammation in the colon and rectum. The incidence and prevalence of UC have been reported to be increasing over the past two decades^[1]. Due to its unknown etiology, high risk of recurrence, and poor prognosis, UC has become a clinical challenge in terms of treatment. Meanwhile, conventional therapies for UC fail to successfully induce remission and prevent relapse, and also possibly cause various side effects. Therefore, studies exploring the alternative therapies for UC have become a topic of great interest.

In recent years, herbal medicine, the most common modality of alternative and complementary treatment,

has been established for the treatment of UC, and the bioactivities of herbs have been explored by taking a bench-to-bedside approach. Intriguingly, combination treatments with traditional Chinese medicine, especially herbs, have shown to exhibit the preferential effect than single conventional treatment for UC^[2], indicating that herb medicine may be a promising alternative treatment for UC in future. In this review, we summarize the potentials of these herbs and their involvement in clinical management of UC.

PATHOGENESIS OF UC

UC is characterized by aberrant innate and adaptive immune responses. Neutrophils, the first line of innate immune cells, are responsible for intestinal tissue damage, through releasing a large amount of toxic components and free radicals upon stimulation, during the progression of UC^[3,4]. Meanwhile, atypical T helper cell (Th) type 2 responses is reported in the pathogenesis of UC, including excessive activation of non-classic natural killer T cells and Th2 cells, as well as substantial production of cytokines, *e.g.*, interleukin (IL)-5 and IL-13. Elevated cytokine levels are noted in UC patients, including IL-5, IL-13, and other proinflammatory cytokines such as tumor necrosis factor (TNF). Once released by immune cells, the cytokines act to further trigger immune responses, induce apoptosis of epithelial cells and upregulate claudin-2 expression, which result in impairment of tight junction of intestinal epithelial cells, and herein damage of the epithelial barrier^[5-7].

Nuclear factor kappaB (NF- κ B) is a transcription factor regulating the expression of a variety of genes, *e.g.*, TNF, in response to extracellular inflammatory stimuli^[8]. Since elevated TNF expression is reported in blood^[9], stool samples^[10], and the mucosa^[11] of patients with active UC, it is widely accepted that NF- κ B plays a pivotal role in the development of UC. The relevance of NF- κ B inhibition in IBD is further demonstrated by treatment of experimental colitis with a NF- κ B antisense oligonucleotide, which resulted in amelioration of inflammation in the colon^[12].

Intestinal microbiota is also suggested to participate in the progression of UC. A recent study has showed that fecal microbiota composition of UC patients varies significantly from healthy subjects, indicating the potentials of microbial alterations in patients with UC^[13]. Intestinal immune cells are tolerant to lumina commensal antigens, but such tolerance is broken as seen in patients with UC and Crohn's disease^[14,15]. The current findings suggest that the defective dynamic balance between commensal microbiota and host defense may contribute to the pathogenesis of UC^[16].

HERBAL MEDICINE: THERAPY FOR UC

Herbal medicine is the traditional Chinese clinical practice using plants or/and plant extracts for medical treatment. Due to lack of desirable efficacy and poor toler-

ance of conventional drugs, more and more populations prefer to accept herb medicine under disease conditions, *e.g.*, headache and infections. Approximately 9.6% to 12.1% of the US adults use one or more forms of herbal products to alleviate disease symptoms, amongst them proximately 10% for digestive symptoms^[17]. Recently, herb medicine is employed in clinical trials for UC treatment in many countries including China and India^[18].

To study the clinical effect of herbal medicine treatment on UC patients, we searched the controlled clinical trials in PubMed, Google Scholar, and Cochrane Trial Register databases. As a result, a total of 9 controlled studies were included regarding the treatment for UC patients by herb medicine. Among them, 5 were randomized, double-blind, placebo-controlled studies, and one was individually controlled cohort study. These herbs/herb extracts used in the clinical trials included *aloe vera* gel, butyrate, tormentil extracts, wheat grass juice, and curcumin, which are mainly summarized in Table 1.

ALOE VERA GEL

The *aloe vera* plant has been used for skin care as well as medicine for centuries. The leaf of the *aloe vera* plant consists of two main parts: an inner central leaf pulp that stores *aloe vera* gel, the bioactive component, and an outer leaf pulp responsible for transportation of *aloe vera* latex. Aloe vera gel becomes well known due to its anti-inflammatory properties, and is under therapeutic evaluation for UC treatment^[19]. For example, *aloe vera* gel inhibits prostaglandin E2 and IL-8 secretion, while having no effect on thromboxane B2 production in the human colorectal mucosa^[20]. *Aleo vera* gel has been further reported to inhibit the release of reactive oxygen species (ROS) by PMA-stimulated human neutrophils, and abrogate the ROS-dependent cytotoxicity of neutrophils such as lysis of red blood cells^[21]. The anti-inflammatory activities of *aloe vera* gel provide the evidence that it may have a therapeutic effect on IBD.

The clinical value of *aloe vera* gel has been assessed. In a randomized, double-blind, placebo-controlled trial, 44 hospitalized patients with mild or moderate UC received oral *aloe vera* gel treatment or placebo, 200 mL daily for 4 wk^[22]. Clinical remission, improvement and response of the disease had been observed in 9 (30%), 11 (37%) and 14 (47%), respectively, of 30 UC patients taking *aloe vera*, compared to one (7%), one (7%), and two (14%), respectively, of 14 UC patients receiving placebo. The clinical colitis activity index and histological scores of the patients decreased significantly during treatment with *aloe vera* ($P = 0.01$ and $P = 0.03$, respectively), but not with placebo. Endoscopic score and laboratory variables displayed no significant differences in both groups of patients with *aloe vera* or placebo treatment. Side events were minimal and similar between *aloe vera* and placebo.

BUTYRATE

Butyrate, a four-carbon short-chain fatty acid, is the main

Table 1 Summary of trials using herbal therapy for patients with ulcerative colitis

Ref.	Herbal medicine	Patient number	Trial design	Treatment method	Duration of treatment	Remission on herb	Remission on placebo
Langmead <i>et al</i> ^[22]	Aloe vera gel	44	Randomized, double-blind, placebo-controlled study	Oral	4 wk	30%	7%
Vernia <i>et al</i> ^[34]	Butyrate	25	Randomized, double-blind, placebo-controlled study	Oral	6 wk	58.3%	38.4%
Huber <i>et al</i> ^[39]	Tormentil extracts	16	Individually controlled cohort study	Oral	3 wk	-	-
Ben-Arye <i>et al</i> ^[42]	Wheat grass juice	23	Randomized, double-blind, placebo-controlled study	Oral	4 wk	Not stated, but wheat grass improved symptoms and bleeding more than placebo	Not stated
Singla <i>et al</i> ^[45]	Curcumin	45	Randomized, double-blind, placebo-controlled study	Enema	8 wk	43.4%	22.7%
Hanai <i>et al</i> ^[46]		89	Randomized, double-blind, placebo-controlled study	Oral	6 mo	95.3%	79.5%

metabolite in the colon derived from bacterial fermentation, and also an important energy source of intestinal epithelial cells. Depletion in butyrate-producing microbial communities has been reported in colon mucosal samples from UC patients, attributing to deficiency of butyrate production and exhaustion of energy supplies to intestinal epithelial cells^[23,24]. Nevertheless, oral supplement of butyrate exhibits anti-inflammation functions, and ameliorates murine colitis, *via* reduction of neutrophil infiltration and attenuation of intestinal inflammation^[25]. Currently, functions of butyrate have been linked with regulation of innate immune responses. For example, butyrate down-regulates lipopolysaccharide-induced expression of proinflammatory mediators by macrophages and neutrophils, including nitric oxide, IL-6, and IL-12, through inhibition of NF- κ B activation and histone deacetylase activities^[26-29]. Butyrate has also emerged as a modulator of adaptive responses, owing to its multiple bio-functions, *i.e.*, restoring transforming growth factor beta and IL-10 production in the colon mucosa, inducing T cell apoptosis and dampening interferon- γ (IFN- γ) secretion^[30].

Clinical trials have shown the effectiveness of butyrate monotherapy or/and in combination with conventional treatment in patients with UC, diversion colitis, as well as acute radiation proctitis^[31-33].

A randomized, double-blind, placebo-controlled pilot study on UC patients was conducted to evaluate the safety and efficacy of oral sodium butyrate tablets, coated with a pH-dependent soluble polymer^[34]. Administration of butyrate (4 gram daily) in combination with mesalazine significantly improved the disease activity score in 25 patients with active UC, in comparison with mesalazine treatment alone. The combined treatments other than mesalazine alone decreased disease activity index score, and significantly improved disease outcomes *vs* baseline values ($P < 0.05$). Meanwhile, the histological and endoscopic scores improved after treatment in both groups ($P < 0.05$). The similar observations were reported in other non-controlled clinical trials using oral administration or enemas of butyrate^[32,35,36].

TORMENTIL EXTRACTS

Tormentil is a member of the rose family that grows wild over Europe. Tormentil extracts contain a high content of tannins which displays potent superoxide-scavenging effects, suggesting tannins as an anti-inflammatory agent. Tormentil has also been shown to be effective in treatment of diarrhea or intestinal inflammation^[37,38]. *In vitro* studies have further confirmed the anti-inflammatory, anti-oxidative, and bacterial growth regulatory effects of tormentil extracts^[37].

Positive results of tormentil extract treatment have been observed in individual patients with UC^[39]. Sixteen patients with active disease took oral tormentil extracts in escalating doses of 1200, 1800, 2400, and 3000 mg every day for three weeks each. Every treatment phase was followed by a 4-wk washout phase. During treatment with 2400 mg of tormentil extracts per day, the clinical activity index, and C-reactive protein levels decreased from 8 mg/L (range: 6-10.75 mg/L) and 8 mg/L (range: 3-17.75 mg/L) at baseline to 4.5 mg/L (range: 1.75-6 mg/L) and 3 mg/L (range: 3-6 mg/L), respectively. During treatment, the clinical activity index improved in all patients, but it turned to increase during the washout phase. There were no apparent side effects with tormentil extract treatment observed during the study.

WHEAT GRASS JUICE

Wheat grass juice is the extract from the pulp of wheat grass and has been used for the treatment of various intestinal diseases and thalassemia for several years. By radical scavenging in correlation with phenolic and flavonoid contents inside, wheatgrass extracts exhibited an antioxidant activity^[40]. In particular, pigenin, the main constituent in wheat grass, was shown to inhibit the production of proinflammatory cytokines, *e.g.*, IL-1 β , IL-8, and TNF in LPS-stimulated human and mouse macrophages, by inactivating NF- κ B through suppression of p65 phosphorylation^[41].

The clinical usage of wheat grass juice in UC treat-

ment has been reported^[42]. In a randomized double-blind placebo-controlled trial, 23 patients with active UC were randomly grouped to receive either 100 mL of wheat grass juice, or the same volume of placebo, daily for 1 mo. Efficacy of treatment was evaluated by disease activity index, bleeding feces, number of bowel movements, sigmoidoscopic evaluation, and global assessment. The patients treated with wheat grass juice showed significant reductions in disease activity index ($P = 0.031$) and severity of rectal bleeding ($P = 0.025$), in contrast to those receiving placebo. No adverse effects of wheat grass juice were observed.

CURCUMIN

Curcumin is an active phytochemical substance in turmeric, and exhibits pharmacologic activities that might benefit patients with UC. A large number of publications have reported the promising pharmacologic effects of curcumin, *i.e.*, inhibition of expression of a variety of inflammatory genes, including cyclooxygenase (COX)-1, COX-2, lipoxygenase, TNF, IFN- γ , inducible nitric oxide synthase, as well as abrogation of NF- κ B activation^[43]. Recently, curcumin has been shown to attenuate colonic inflammation through direct inhibition of neutrophil chemotaxis and chemokinesis, and partly through inhibition of the chemokine expression^[44].

Clinical trials have evaluated the therapeutic effect of curcumin in patients with mild-to-moderate UC. In a randomized, double-blind, single-centre pilot study, 45 patients received oral 5-aminosalicylic acid in combination with either curcumin preparation (140 mg in 20 mL water) or placebo enema. The patients receiving additional curcumin preparation treatment showed improvements in disease activity, compared with those patients with placebo enema^[45]. Another group also showed the similar efficacy of combination treatment of curcumin (2 g daily) and sulfasalazine or mesalamine in maintenance therapy for 89 patients with quiescent UC, indicating that curcumin may confer additional therapeutic advantages when used in combination with conventional anti-inflammatory medications in UC^[46].

SAFETY OF HERB MEDICINE

So far, it remains unclear about the safety of herb medicine. Butyrate, the most common treatment used for UC patients, has been shown to be relatively safe for UC patients. Hallert *et al.*^[47] reported that supplement of dietary fiber elevated the fecal butyrate level, and kept UC patients in remission, without increment in gastrointestinal complaints during the trial. Recently, a meta-analysis evaluated the efficacy and tolerance of herbal medicines in patients with IBD. With the results from seven placebo-controlled clinical trials, the analysis has showed that herbal medicines can induce clinical response and remission in IBD patients, without serious side events^[48].

Due to limitation of human studies, animal models

become alternatives to explore the safety of herbs. Acute toxicity of Tormentil rhizomes was assessed in rats and mice, with a single dose administration by gavage of 2.5 and 6.8 g/kg (body weight), respectively^[49]. No apparent toxic effects have been recorded at two weeks after the administration of Tormentil rhizomes. Nevertheless, some researchers questioned the safety of herbs with the evidence that fatal hepatic and irreversible renal failure occurred with some herb preparations, and that interactions of herbs with conventional drugs were potentially detrimental^[50]. Meanwhile, a recent study has reported the increased incidences of mucosa hyperplasia and goblet cell hyperplasia in the colon of rats and mice at 13 wk after exposure to drinking water containing *aloe vera*^[51]. Thus, the safety and long-term benefits of herb medicine need to be intensively investigated before it can be applied for patients.

CONCLUSION

Because of the relatively natural and multiple biological properties, herbs have emerged as the alternative for current treatment of inflammatory disorders, including UC. Clinical trials have indicated the promising possibility of herb medicine for UC treatment. However, there have some concerns to be clarified before herb medicine can be securely introduced into UC patients. So far, the clinical trials with herb medicine treatment were conducted in a small number of UC patients, and large case-controlled studies and reliable data about the detailed mechanism of the herbs are still lacking. Meanwhile, herbal preparations are the mixture containing a huge range of biological compounds, other than purified single component. It might not be known which component in the herbs provides the exact pharmacological effects, even in some cases the herb mixtures exhibit clinical benefits. Thus, determination of herb components, dosage and course of herb treatment becomes a challenge for clinical employment. In addition, the safety of herb medicine remains to be further investigated, especially under long term treatment.

Overall, herb medicine treatment becomes widespread and prevalent, with encouraging results from clinical trials. Further evidence about the components of herbs and their bio-functions will shed light on clinical administrations of herb medicine in future. With discerned safety of herbs, herb medicine itself or in combination with conventional therapies would largely benefit patients with UC and other immune disorders.

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Predictors of recurrence of Crohn's disease after ileocelectomy: A review

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Abstract

Recurrence after ileocelectomy for Crohn's disease (CD) is common and occurs in up to 80% of patients. Such recurrence can result in repeated surgical interventions, an increased need for medical treatment and, frequently, an impaired quality of life. The aim of this overview is to provide a summary of the factors associated with disease recurrence after ileocelectomy for CD. Recurrence can be measured clinically or endoscopically using established scoring systems. Radiology and serologic tests can also be used, oftentimes in conjunction with endoscopy and/or clinical findings. Many patient and operative factors as well as pharmacologic treatments have been studied as potential predictors of recurrence. Of these, only smoking and immunomodulatory or biologic medical treatment have repeatedly been shown to effect recurrence. Genetic predictors have been studied and suggested but further evaluation in larger cohorts is necessary. This paper highlights validated, reproducible scoring systems for recurrence and the key findings of studies including patient demographics, operative techniques, various pharmacological treatments and histological findings

as predictors of recurrence post ileocelectomy in CD.

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Key words: Crohn's disease; Ileocelectomy; Recurrence; Surgical genetics; Inflammatory bowel disease

Core tip: Disease recurrence after ileocelectomy for Crohn's disease is common. Studies have been very heterogenous in defining recurrence as it can be clinical, endoscopic, radiologic or serologic. Of the potential predictive factors studied, smoking has been consistently demonstrated to increase the risk of recurrence. While immunomodulator and biologic medical treatment have been shown to increase the time between surgery and recurrence and may decrease overall risk. Genetic predictors have been suggested but further evaluation in large groups is needed. Several other demographic and operative factors have been studied. However, none have been consistently shown to affect recurrence risk.

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INTRODUCTION

Crohn's disease (CD) is a chronic immune mediated disease of the gut that was first described as "regional ileitis" by Crohn, Ginzburg and Oppenheimer in a case series presented at American Medical Association annual meeting in 1932^[1]. CD is characterized by inflammation, abscesses, fistulization and stricturing that can affect any part of the gastrointestinal tract. However, the most common distribution is the ileocolic region, the location where the small

Table 1 Types of postoperative recurrence and evaluation type

Type of recurrence	Evaluation method
Clinical	Questionnaire, CDAI, Harvey-Bradshaw Index, IBDQ
Endoscopic	Rutgeerts score, Crohn's disease endoscopic index of severity
Radiographic	CT or MR enterography, barium enema small bowel follow through
Serological	Measurement of CRP and ESR
Surgical	Requirement for repeat surgery

CDAI: Crohn's disease activity score; IBDQ: Inflammatory Bowel Disease Questionnaire; CT: Computed tomography; MR: Magnetic resonance; CRP: C reactive protein; ESR: Erythrocyte sedimentation rate.

Table 2 Factors in the Crohn's disease activity score

General well-being
Number of stools/d
Abdominal pain
Weight loss
Presence of arthralgia, fistuli, fever and/or ocular, dermatological or anal manifestations
The need for anti-diarrheal medication
Abdominal mass
Hematocrit

Based on patient symptoms during the 7 d prior to taking the survey.

Table 3 Factors in the Rutgeerts endoscopic recurrence score for postoperative recurrence of Crohn's disease in the distal ileum

Endoscopic appearance	Score
No aphthous ulcers	0
< 5 aphthous ulcers	1
> 5 aphthous ulcers with normal mucosa between the ulcers	2
Diffuse aphthous ulcers throughout the ileum with intervening inflamed mucosa	3
Large ulcers with diffuse inflammation, nodules or narrowing of the ileum	4

bowel and colon meet. Approximately 55% of all CD patients have an ileocolic disease distribution, followed by colonic and small bowel distributions in approximately 20%-30% and 15%-20% and of patients respectively^[2].

Although not curative, surgery is commonly required for the sequelae CD (*e.g.*, abscess, fistula, perforation, bleeding and failure of medical treatment). Up to 80% of CD patients require at least one surgical intervention in their lifetime. The most common resection is the ileocelectomy. Recurrence at the site of the anastomosis is common and challenging^[3,4]. Multiple resections due to recurrent disease can lead to short gut syndrome, malabsorption and malnutrition with significant morbidity, decreased quality of life and increased hospital and outpatient costs^[5]. This review highlights the patient and disease related factors that are associated with an increased risk of disease recurrence after ileocelectomy in its many forms including clinical, endoscopic and radio-

logic recurrence.

Defining recurrence

Recurrence can be defined in several different ways using a multitude of modalities. Such inconsistency in regard to what constitutes recurrence in conjunction with heterogeneity among patient populations and prophylactic measures against recurrence given leads to a large variance in recurrence rates between studies (Table 1)^[6]. Clinical and endoscopic recurrence are most commonly reported.

Clinical recurrence is loosely defined as an increase in patients' symptoms including diarrhea, weight loss and abdominal pain. For the most appropriate investigation and reporting of clinical recurrence, established validated quality of life questionnaires such as the Inflammatory Bowel Disease Questionnaire^[7] or scoring systems such as the Crohn's disease activity score (CDAI) or Harvey-Bradshaw Index should be used^[8]. The CDAI can be clinician or self-administered and contains subjective questions (on general well-being and symptoms such as abdominal pain) as well as objective measures (such as hematocrit, numbers of stools per day, weight loss, the presence of arthralgia, fistuli, fever, an abdominal mass and/or ocular, dermatological or anal manifestations) (Table 2)^[9,10]. Endoscopic recurrence uses colonoscopy or ileoscopy as appropriate to determine the presence of recurrence, which is most commonly found at the site of the anastomosis. The Rutgeerts visual grading system evaluates the presence and number of aphthous ulcers and the intervening mucosa in the perianastomotic region and is the most commonly used internationally recognized endoscopic grading system for post ileocelectomy recurrence (Table 3)^[11,12]. Radiographic recurrence is less commonly studied and is often utilized as an adjunct to clinical or endoscopic recurrence. CT or MR enterography, small bowel follow through and/or barium enema are the modalities currently employed^[13]. Serological recurrence is defined by the elevation of serum inflammatory markers such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Although more novel interleukin markers have been studied, none are in clinical use to date. Surgical recurrence is determined by the requirement for repeat ileocelectomy and is often indicative of more severe disease. Oftentimes, these different classifications of recurrence are studied in conjunction with each other.

Endoscopic, radiographic and serologic recurrence rates are in actuality higher than reported as frequently, only clinically symptomatic patients are investigated. Additionally, there is limited concordance between the different types of recurrence in the individual patient. For example, Bordieianou *et al*^[14] compared endoscopic, symptomatic and surgical recurrence rates in approximately 200 ileocelectomy patients and found that while 31.2% of their cohort had documented endoscopic recurrence, only 23.1% had symptomatic and 11% had surgical recurrence. This disparity was also demonstrated

Table 4 Overall recurrence rates by post ileocelectomy follow up and type of recurrence

Time post ileocelectomy	Type of recurrence	% of ileocelectomy Patients	Ref.
1 yr	Clinical	0%-44%	McLeod <i>et al</i> ^[61] , Walters <i>et al</i> ^[10] , Aratari <i>et al</i> ^[27] , Bordeianou <i>et al</i> ^[14] , Sorrentino <i>et al</i> ^[28] , Pascua <i>et al</i> ^[53]
	Endoscopic	0%-84%	Bordeianou <i>et al</i> ^[14] , Walters <i>et al</i> ^[10] , McLeod <i>et al</i> ^[15,61] , Regueiro <i>et al</i> ^[16,82] , Rutgeerts <i>et al</i> ^[11] , Pascua <i>et al</i> ^[53] , Domènech <i>et al</i> ^[80] , Sorrentino <i>et al</i> ^[28] , Meresse <i>et al</i> ^[40] , Lasso <i>et al</i> ^[89]
	Surgical	4%-25%	Aratari <i>et al</i> ^[27] , Iesalnieks <i>et al</i> ^[57]
5 yr	Clinical	32%	Aratari <i>et al</i> ^[27]
	Endoscopic	55%-77%	Bordeianou <i>et al</i> ^[14] , McLeod <i>et al</i> ^[15] , Yamaoto <i>et al</i> ^[42]
	Symptomatic Surgical	50% 4%-25%	Bordeianou <i>et al</i> ^[14] , Bordeianou <i>et al</i> ^[14] , Aratari <i>et al</i> ^[27] , Riss <i>et al</i> ^[56] , Yamamoto <i>et al</i> ^[42]
10 yr	Clinical	52%	Aratari <i>et al</i> ^[27]
	Endoscopic	74%	Malireddy <i>et al</i> ^[17] , Bordeianou <i>et al</i> ^[14]
	Surgical	12%-57%	Stocchi <i>et al</i> ^[18] , Aratari <i>et al</i> ^[27] , Riss <i>et al</i> ^[56] , Iesalnieks <i>et al</i> ^[57]

in McLeod *et al*^[15] 1997 study which included a variety of CD resections, of which 60% were ileocelectomies. Interestingly, 21% of patients with severe symptoms had minimal endoscopic or radiologic evidence of recurrence. Conversely, 28% of the asymptomatic patients studied had endoscopic or radiologic evidence of severe recurrence. Similarly, Regueiro's study of 24 CD patients 1 year post ileocelectomy demonstrated a poor correlation between CDAI scores, serum CRP or ESR and endoscopy findings^[16]. Only 87% of patients with endoscopic or radiologic recurrence had symptoms in Malireddy's study^[17].

Endoscopic recurrence generally occurs earlier than other types of recurrence with an overall mean time from surgery to endoscopic recurrence ranging from 6 mo to 4 years^[14,17]. Mean time to symptomatic recurrence is approximately 5 years^[14]. Mean time to repeat surgery has the longest duration, approximately 7 (range of 5-11) years^[9]. Overall, rates of approximately 30% for endoscopic, 23% for symptomatic or clinical and 11%-50% for surgical recurrence are documented in the literature^[3,14,18]. However, it is more clinically relevant to evaluate recurrence by time period, according to time since ileocelectomy. Overall recurrence rates by time period are shown in Table 4. The multiple factors affecting recurrence are discussed below.

Table 5 Effect of smoking on postoperative recurrence

Association	Number and type of patients	Ref.
Recurrent clinical symptoms (OR = 2.96)	59 patients post colonic resection	Kane <i>et al</i> ^[22]
Shorter duration to clinical relapse (104 wk shorter)	for CD (not only ileocelectomies)	
Recurrent clinical symptoms (worse CDAI scores)	182 post colonic resection for CD	Cottone <i>et al</i> ^[23]
Increased rates of endoscopic recurrence	(not only ileocelectomies)	
Increased likelihood of requiring surgery		
Smoking at the time of the 1 st ileocelectomy conferred a 2.1 fold increased likelihood of requiring another operation	176 post ileocelectomy patients with at least 1 recurrence	Unkart <i>et al</i> ^[21]
OR of 2.2 for clinical recurrence	Meta-analysis of 16 studies, 2962 patients	Reese <i>et al</i> ^[24]
Increased risk of surgical recurrence particularly at 10 years (OR = 2.6)		
Smokers had a lower 5 and 10-yr recurrence free likelihood (65 and 45% vs 81 and 64% in nonsmokers)	141 ileocelectomy patients	Yamamoto and Keighley ^[26]
Recurrence free rates were lower in those that smoked > 15 cigarettes per day		
Patients that quit smoking are less likely to require redo ileocelectomy	266	Ryan <i>et al</i> ^[25]
No association with recurrence	89 lap ileocelectomy patients	Malireddy <i>et al</i> ^[17]
No association with clinical or surgical recurrence	83	Aratari ^[27]
No association with clinical or endoscopic recurrence	43 resections (30 = Ileocelectomies)	Sorrentino ^[28]

CD: Crohn's disease; CDAI: Crohn's disease activity score.

PATIENT FACTORS

Demographics

Although gender, age at diagnosis, age at surgery and disease duration have been studied as potential predictors of post-operative recurrence, no correlation has been consistently demonstrated^[14,17,19,20].

A positive family history of IBD was demonstrated to confer a 2.2 fold increased likelihood of requiring a second ileocelectomy in Unkart's study of 176 post ileocelectomy patients with at least one surgical recurrence. However, this result has not been replicated in the literature^[21].

The most studied and most recognized risk factor for post-operative recurrence is smoking (Table 5). Several studies evaluating smoking as a predictor of recurrence do not exclusively study ileocelectomies. One such study by Kane *et al*^[22] followed 59 CD patients post colonic resection. Sixty-nine percent of smokers vs 23% of nonsmokers had recurrence documented using a clinical symptom activity score. Odds ratio for recurrence was 2.96 in the smoking cohort and a strikingly shorter dura-

tion to clinical relapse was seen in smokers (130 *vs* 234 wk in nonsmokers). Cottone *et al.*^[23] similarly followed 182 surgical CD resection patients for 6 years and demonstrated that both smoking and greater disease extent were associated with worse clinical (CDAI) scores and increased risk of endoscopic recurrence. Although several variables were studied, the only significant predictor of surgical recurrence was smoking. Meta-analysis of 16 studies published between 1966-2007 inclusive of 2962 patients undergoing resection for CD demonstrated an OR of 2.2 for clinical postoperative recurrence in smokers and an increased risk of surgical recurrence particularly at 10 years (OR = 2.6, 55.5% *vs* 32.1% in nonsmokers)^[24]. However, again, this analysis was not limited to patients who had undergone ileocectomies only.

Studies that focus solely on ileocectomy recurrence include Unkart *et al.*^[21] study which demonstrated that smoking at the time of the 1st ileocectomy confers a 2.1 fold increased likelihood of requiring another operation. http://www.ncbi.nlm.nih.gov/pubmed?term=Unkart%20J%5BAuthor%5D&cauthor=true&cauthor_uid=18536967 Repeat ileocectomy rates of 59%-69% in smokers have been noted by Cullen *et al.*^[31] and Ryan *et al.*^[25]. Another study evaluated the relationship between the number of cigarettes smoked daily and recurrence in 141 ileocectomy patients. Smokers had lower 5 and 10 year recurrence free likelihoods (65% and 45 % *vs* 81% and 64% in non-smokers). Recurrence free rates were lower in those that smoked > 15 cigarettes per day^[26]. Although the majority of studies have shown an increased risk for reoperation, Maliredu, Aratari and Sorrentino did not see such an association in their studies which included approximately 195 patients combined^[17,27,28].

Genetics

Since the advent of genome wide associations studies, several studies inclusive of large cohorts of both IBD patients and healthy individuals have been performed thus creating a pool of IBD-associated genes and single nucleotide polymorphisms (SNPs). To date, over 300 SNPs and 150 genetic loci have been associated with IBD^[29]. With these IBD-associated genes established, a shift towards using these markers to further characterize disease behavior, including postoperative recurrence in CD, has begun^[30]. Several "surgical genetics" studies have identified markers of the need for resection in their CD cohorts including mutations within the NOD2, TNFSF15 and C13ORF31 genes^[31]. However, fewer studies have focused on determining a marker of recurrence.

NOD2 (nucleotide-binding oligomerization domain-containing protein 2), also known as CARD15 (caspase recruitment domain-containing protein 15), was the first gene to be associated with IBD in 2001^[32]. Located on chromosome 16, the gene is expressed in several different cell types key to the pathogenesis of CD such as dendritic cells, monocytes, intestinal epithelial cells and Paneth cells. Its protein product is involved in the rec-

ognition a dipeptide found in the bacterial cell wall^[33]. NOD2 has been previously associated with ileal^[34] and stricturing CD^[35].

An early German study evaluated the NOD2 genotypes of 51 post ileocectomy patients. Fourteen patients required a repeat ileocectomy. Of the 14, 12 harbored at least 1 NOD2 mutation^[36]. This association may be specific to patients of German and other not yet determined ethnicities, as this increased incidence of NOD2 mutations in patients with recurrent disease was not replicated in an Italian multi-center study of 253CD patients, 42% of whom had ileocolic disease. In this study, no relation between NOD2 genotype, age at diagnosis or smoking status and recurrence was found^[37]. Similarly, in a recent meta-analysis of 6 studies inclusive of 1003 patients with CD NOD2 genotype was not associated with surgical recurrence. Overall, 39% of patients with a NOD2 mutation required further resection *vs* 30.5% of patients without a mutation (*P* = 0.06). However, the included studies were very heterogenous, which may have affected results^[38].

Another study of only ileocectomy patients demonstrated the presence of a mutation in the autophagy associated IRGM (Immunity-related GTPase family, M) gene to be significantly associated with more frequent ileocectomies and earlier time to reoperation in 66 CD patients. Ileocectomy was performed every 6.8 +/- 1.3 years on average in patients with the at risk genotype for SNP rs4958847 *vs* once every 11.4 years in patients with the wild type genotype^[39].

Meresse *et al.*^[40] studied G microsatellite genotype with the anti-inflammatory gene, IL10 in 36 post ileocectomy patients. Although genotype affected IL-10 production, no association was seen with endoscopic recurrence.

Nutritional status

Poor nutritional status has been consistently associated with poor outcomes in CD surgery^[41]. Thus postoperative enteral feeding has been studied as a potential way to reduce the risk of complications and recurrence. Often, such studies are technically difficult to perform, particularly after patients are discharged from the hospital, and mid-study patient exclusion due to noncompliance is common. In one such Japanese study of 40 post ileocectomy patients followed for 5 years, 20 received nighttime continuous nasogastric feeding with an elemental diet and a low fat diet during the day. The other 20 did not receive the nighttime feed and had an unrestricted diet. No postoperative steroid, immunosuppressants or biologics treatment was given to either cohort. Thirty percent of the nighttime feeding group *vs* 70% of the controls had endoscopic recurrence at 1 year. Twelve months after surgery, 10% of the night feed and 45% of the control group required biologics during the follow up. Rates for surgical recurrence were 5% and 25% in the 2 groups but this was not statistically significant^[42].

Much attention has been given to the gut microbi-

ome and how it interacts with the disease process in CD^[43]. However, few studies have centered on altering the gut microbiome to maintain remission in patients who have undergone resection. A meta-analysis of 10 controlled clinical trials evaluating disease recurrence, demonstrated that probiotics were not associated with either endoscopic or surgical remission. In this meta-analysis patients who had undergone surgery or medical treatment prior to the administration of probiotics were not considered separately^[44]. Another systematic review of 24 manuscripts evaluating endoscopic recurrence demonstrated that enteric diets were associated with 61% reduction in endoscopic scores. However, again postoperative patients and patients in medical remission were not considered separately^[45]. One multicenter randomized study by Van Gossum *et al*^[46] focused only on post ileocelectomy patients and the effect of administration of the probiotic LAl *vs* placebo on early (12 wk) post ileocelectomy recurrence in 49 patients. Patients were stratified according to smoking status. There was no difference in endoscopic or clinical recurrence scores seen between the 2 groups. Twenty one percent of the placebo *vs* 15% of the probiotic group had recurrence scores indicative of severe recurrence but this difference was not statistically significantly different.

DISEASE BEHAVIOR

Penetrating vs non-penetrating

Disease behavior is difficult to use as a predictor of endoscopic recurrence due to the varying course of CD typically seen within the individual patient. The Montreal classification of inflammatory *vs* stricturing *vs* penetrating (fistulizing/abscessing) disease is commonly used to classify CD according to its behavior^[47]; however, behavior often changes from inflammatory to stricturing or penetrating over time^[48-50]. Also, at any time point a patient may have more than 1 type of disease behavior, (*i.e.*, a stricture and a fistula) presenting challenges with classification. Additionally, penetrating disease that is initially responsive to a particular medical treatment may lose responsiveness with repeated doses and thus transform from a penetrating to inflammatory to penetrating phenotype when responsiveness ceases^[51].

Nonetheless, several studies have attempted to correlate disease behavior with risk of post-operative recurrence. In multiple studies, including 2 large Italian multicenter studies, disease behavior was not shown to affect the risk of disease recurrence clinically, surgically or endoscopically^[14,20,27,52]. Although not a primary end point of the study, a meta-analysis of 12 randomized controlled trials that evaluated medical therapies after CD resections (not exclusively ileocelectomies) from 1966 to 2005, demonstrated that fistulizing disease was significantly associated with endoscopic but not clinical recurrence in patients who had been given placebo treatment^[53].

Granulomatous disease

Granulomas, or histologic areas of macrophage fusion,

are a hallmark of CD but need not be present for a definitive diagnosis^[54]. An association between granulomas and disease severity has been suggested but not proven^[55]; however, an association between the presence of granulomas in the ileocolic resection specimen and recurrence has been consistently demonstrated. In an observational study by Malireddy *et al*^[17] recurrence rates in 89 patients who had undergone laparoscopic ileocelectomy from April 1994-August 2006 (with a median follow up of 3.5 years) were evaluated. A 61% endoscopic, radiologic or pathologic recurrence rate was noted. The median time for recurrence was 13.1 mo (range, 1.3 mo to 8.7 years). Several potential prognosticators for recurrence including postoperative biologic and other medical treatment were also studied. The only significant predictor of recurrence found on multivariate analysis was the presence of granulomas in the initial resection specimen. An earlier Irish study of 139 patients who underwent ileocelectomy between 1980 and 2000 evaluated the presence of symptoms, endoscopic recurrence and radiological recurrence. Again, the presence of granulomas in the specimen was significantly associated with **clinical and surgical** recurrence^[3]. In a similar early study of 114 ileocelectomy patients, 66% with granulomas *vs* 48% without experienced an endoscopic recurrence within the first year after an ileocelectomy^[11]. In 1997, Anselme *et al*^[20] evaluated 130 CD patients undergoing a variety of resections. After multivariate analysis, the presence of granulomas was significantly associated with recurrence.

INDEX ILEOCELECTOMY DETAILS

Urgent vs elective resection

Several groups have hypothesized that if a patient's first ileocelectomy is performed under emergency circumstances, this likely reflects more severe disease and thus may predict an increased likelihood of recurrence. One retrospective multicenter Italian review evaluated clinical recurrence (defined as the need for steroids in conjunction with endoscopic or radiologic findings) and surgical recurrence in 83 CD patients who underwent ileocelectomy for severe disease at the time of diagnosis *vs* 124 who underwent surgery later in their disease for medical refractory disease and/or complications secondary to their disease. Recurrence was evaluated at 1, 5 and 10 years. Clinical recurrence was less frequent in the early surgery group at all-time points. No difference was seen in the need for or timing of repeat surgery between the 2 groups^[27]. Another retrospective study of 116 consecutive patients undergoing their first ileocelectomy at a large Austrian referral center between 1997 and 2006 demonstrated that urgent index ileocelectomy increased the risk of repeat surgery approximately 6 fold^[56].

Sepsis

Another potential marker of severe disease is perioperative sepsis at the time of index ileocelectomy. However, results are conflicting and further study is warranted. Elevated white cell count was associated with endoscopic

recurrence on univariate analysis in Caprilli *et al.*^[52] study of 110 ileocelectomy patients. However, an increased risk of recurrence in urgent *vs* elective ileocelectomy patients was not demonstrated. Iesalnieks *et al.*^[57] studied 282 patients who underwent 331 varied CD resections between 1992-2005. On multivariate analysis, postop intraabdominal septic complications and history of a previous resection were associated with increased surgical recurrence risk at all-time points studied from 1-10 years. At 1 year patients with a history of sepsis had a recurrence rate of 25% *vs* 4% of those without sepsis. At 10 years rates were 57% and 38% respectively.

Laparoscopic vs open approach

As laparoscopic surgery and stapled anastomosis initially gained popularity for use in the CD population in the 1980s, an interest in determining if either technique decreases the risk of recurrence post ileocelectomy led to several studies. One such retrospective review of 113 patients undergoing their index ileocelectomy between 1987-2003 (with a mean follow up of approximately 70 mo) demonstrated a slightly lower, but not significantly different, rate of postoperative medical treatment requirement in laparoscopic *vs* open patients (39% *vs* 54%). Surgical recurrence was seen in 9.5% (6/63) of laparoscopic *vs* 24% (12/50) of open ileocelectomy patients during a mean follow up of 81 mo. Time to recurrence was not affected. Both groups had a median time to recurrence of approximately 60 mo^[58]. Similarly, Stocchi *et al.*^[18] performed a prospective randomized trial in which 77 patients underwent laparoscopic ileocelectomy and 29 underwent an open procedure. Both cohorts had a similar postoperative prophylactic medication regimen and a mean follow up of 10.5 years. Reoperation rates were the same in the 2 groups (approximately 26% in each). Endoscopic and radiologic recurrence rates were similar. Forty-eight percent of laparoscopic patients experienced endoscopic or radiologic recurrence *vs* open patients who demonstrated a 66% endoscopic recurrence and 52% radiologic recurrence rate. The most recent meta-analysis on laparoscopic *vs* open approach included 33 studies and included 2519 patients. No statistical difference was seen in surgical recurrence rates which were 25 per 1000 person years in the laparoscopic group *vs* 34 per 1000 person years in the open group^[59].

Resection margins and type of anastomosis

Stapled anastomosis has now virtually replaced the hand sewn technique in the majority of centers performing ileocelectomies for CD. Two studies, inclusive of 199 and 89 ileocelectomy patients, evaluated resection margins and anastomotic type. In both studies, no difference in clinical recurrence was found between patients whose resection margins were affected by disease *vs* those who had unaffected margins. There was also no difference seen between stapled or hand sewn anastomoses^[14,17]. In the only randomized study to date, Fazio *et al.*^[60] evaluated recurrence in 131 ileocelectomy patients random-

ized to undergo resections with proximal margins either 2 or 12 cm from the macroscopically diseased tissue followed up for median of 56 mo. The resection specimen was also studied for microscopic signs of disease. Surgical recurrence was found in 25% of patients who had undergone a limited resection *vs* 18% of those who had undergone the more extensive resection ($P > 0.05$). Clinical recurrence was demonstrated in 33% *vs* 29% of those with limited and extended resections respectively. No relation was seen between microscopic CD found at the resection margin and recurrence.

In Bordeianou's study of approximately 200 ileocelectomies, stapled anastomosis although significant on univariate analysis, lost significance on multivariate analysis^[14]. End-to-end *vs* side-to-side anastomotic techniques has also been evaluated in 2 randomised trials comparing anastomosis types in 98 and 139 CD patients respectively. Both studies failed to demonstrate a difference in symptomatic or endoscopic recurrence rates between the groups. The first study demonstrated an endoscopic recurrence rate of 42.5% in the end-end *vs* 37.9% in the side-side anastomosis groups and a symptomatic recurrence rate of approximately 22% in both groups^[61]. The second study demonstrated that overall, on multivariate analysis, anastomosis type did not affect endoscopic recurrence. However, a 3 fold risk of recurrence was seen in a subgroup of patients with end-end anastomoses who were treated with 5 aminosalicylates (ASAs). This increased risk was not seen in those not treated with ASAs^[52]. Meta-analysis of 8 studies published between 1992-2005 inclusive of 661 ileocelectomy patients compared end-to-end anastomosis *vs* other anastomotic configurations (stapled side-to-side, end-to-side or side-to-end, stapled circular end-to-end). No significant difference was found in clinical or surgical recurrence between the different groups^[62].

Techniques to potentially minimize risk of recurrence are currently under development. The Kono-S is one such novel technique. This technique utilizes a linear stapler-cutter to transversely divide the tissue for resection. The corners of the 2 stapled lines are sutured together and antimesenteric longitudinal enterotomies are created on both sides. The enterotomies are then closed transversely in two layers resulting in an anti-mesenteric functional end-to-end anastomosis. This technique has shown promise in a small cohort of 18 patients, 43% of whom have undergone follow-up endoscopic surveillance with an average Rutgeert's score of 0.7 (0-3) at a mean of 6.8 mo^[63].

MEDICAL TREATMENT TO PREVENT DISEASE RECURRENCE

Key studies on the effect of medical treatment for the prevention of recurrence are highlighted in Table 6. Traditionally, treatment paradigms for CD followed a "bottom up" approach with initial treatment comprised of corticosteroids, antibiotics and/or 5 ASAs. Escalating

Table 6 Key studies on medical treatment for the prevention of postoperative recurrence in post ileocelectomy patients

Interventions Compared	Study Design	Study Numbers (end of follow-up)	Follow-up	Clinical Improvement	Endoscopic Improvement	Other	Ref.
Mesalamine <i>vs</i> Placebo	Double Blind, Multicenter	87	12 mo	59% of placebo <i>vs</i> 41% of mesalamine had a clinical relapse	Significantly less severe and less frequent lesions in mesalamine group ($P < 0.008$)	Severe endoscopic or radiologic was 24% in mesalamine <i>vs</i> 56% of placebo ($P = 0.004$)	Brignola <i>et al</i> ^[68]
Budesonide <i>vs</i> placebo	Double-blind, randomized trial	129	12 mo	No difference in CDAI at any time point in the study	Only patients who underwent surgery for increased disease symptoms (not fibrostenotic or fistulizing disease) had a significantly lower endoscopic recurrence rate (32% <i>vs</i> 65% of the placebo group)	AT 12 mo the ESR value was 13.3 mm/h in the budesonide group <i>vs</i> 20.2 mm/h in the placebo group ($P = 0.017$). Mean CRP values after decreased from 19.0 to 6.2 mg/L in the budesonide group and from 12.7 to 12.2 mg/L in the placebo group ($P = 0.018$)	Hellers <i>et al</i> ^[64]
Mesalamine <i>vs</i> placebo	Double-blind, placebo controlled ^{1,2}	246	48 wk	25% of the mesalamine <i>vs</i> 36% of the placebo had a relapse [(per CDAI) $P = 0.06$] On subgroup analysis ileocolonic patients had fewer relapses on mesalamine (21% <i>vs</i> 41%) $P = 0.003$		10% <i>vs</i> 23% surgical recurrence ($P = 0.13$)	Sutherland <i>et al</i> ^[67]
Mesalamine <i>vs</i> placebo	Randomized	163 post-surgical patients ¹ 109 were post ileocelectomy	Maximum 72 mo		Endoscopic and radiological recurrence was significantly decreased in the mesalamine group with relative risks of 0.6 ($P = 0.016$)	31% symptomatic recurrence rate (symptoms plus endoscopic and/or radiological confirmation of disease) <i>vs</i> 41% in the control group, $P = 0.03$	McLeod <i>et al</i> ^[69]
6 MP, mesalamine or placebo	Multi (5) center, double blind, randomized	131	24 mo	Clinical recurrence was improved by mesalamine or 6 MP. Clinical recurrence rates at 24 mo were 50% for 6 MP, 58% for mesalamine and 77% for placebo ($P = 0.04$)	Only 6 MP, not mesalamine was superior to placebo to prevent endoscopic and radiographic recurrence at 24 mo. Relapse was 43% with 6 MP, 63% with mesalamine, 64% with placebo ($P = 0.03$)	Radiographic recurrence rates were 33% for 6 MP, 46% for mesalamine and 49% for placebo ($P > 0.05$)	Hanauer <i>et al</i> ^[76]
Infliximab <i>vs</i> mesalamine (control)	Prospective, multicenter pilot study to determine if giving infliximab after diagnosis of postoperative endoscopic ileocolic CD recurrence at 6 mo can induce endoscopic remission at 54 wk	24 (19 had ileocaecal disease)	54 wk	No clinical recurrence in the infliximab group at 6 mo 18% of mesalamine who had clinical relapse by 9 mo	No endoscopic remission at 54 wk in the mesalamine group <i>vs</i> the infliximab group 54% had endoscopic remission at 54 wk ($P = 0.01$)		Sorrentino <i>et al</i> ^[28]
Adalimumab <i>vs</i> AZA <i>vs</i> mesalamine	Randomized	51	2 yr		The ADA treated patients had the lowest incidence of endoscopic recurrence (6.3% <i>vs</i> 64.7% of the AZA group and 83.3% of the mesalamine group)		Savarino <i>et al</i> ^[83]

Infliximab <i>vs</i> placebo	Randomized	24	1 yr	Clinical remission was higher in the IFX group (80% <i>vs</i> 54%) but $P = 0.38$	Endoscopic and histologic recurrence was significantly lower at 1 yr in the patients treated with infliximab (1 of 11; 9.1% and) <i>vs</i> placebo (11 of 13 patients; 84.6%). $P = 0.0006$	Lower histologic recurrence in the IFX group (3 of 11/27% <i>vs</i> 11 of 13/85% of placebo) $P = 0.01$	Regueiro <i>et al</i> ^[82]
Metronidazole +AZA or placebo	62	Randomized	12 mo	Endoscopic recurrence was observed in 14 of 32 (43.7%) patients in the AZA group and in 20 of 29 (69.0%) patients in the placebo group at 12 mo post-surgery ($P = 0.048$. At 1 yr 21% of the AZA group were lesion free <i>vs</i> 3% of the placebo ($P = 0.04$)			D'Haens <i>et al</i> ^[77]
Metronidazole <i>vs</i> placebo	Double-blind controlled	51	3 yr	Clinical recurrence rates at 1 yr were 4% in the metronidazole <i>vs</i> 25% of placebo) NSD $P = 0.04$. Reductions at 2 yr (26% <i>vs</i> 43%) and 3 yr (30% <i>vs</i> 50%) both NSD	At 12 wk, 21 of 28 patients (75%) in the placebo group had recurrent lesions in the neoterminal ileum <i>vs</i> 12 of 23 patients (52%) in the metronidazole group ($P = 0.09$)		Rutgeerts <i>et al</i> ^[65]
Immunosuppressants (AZA/6 MP or MTX) <i>vs</i> control (5 ASAs or no treatment)		26 patients undergoing their 2 nd ileocelectomy	3 yr	Clinical recurrence was lower in the immunosuppressant group <i>vs</i> the control group (3/12, 25% <i>vs</i> 6/10, 60%; $P < 0.05$)		The control group required a 3 rd resection more commonly. (7/12, 58% <i>vs</i> 2/14, 17% $P < 0.02$)	Alves <i>et al</i> ^[79]
AZA therapy commenced immediately post resection	Prospective, observational	56 consecutive patients 15 or 27% had ileocelectomies	Mean 12-84 mo	No clinical recurrence at 12 mo	70% had endoscopic recurrence at 12 mo. The cumulative probability of endoscopic recurrence was 82% at 5 yr		Domènech ^[90]

¹Study included non ileocelectomy patients in addition to ileocelectomy patients; ²Study included medically treated patients in addition to ileocelectomy patients. AZA: Azathioprine; 6 MP: 6 mercaptopurine; ASAs: Aminosalicylates.

treatment in the form of immunomodulators or biologics either replaced this treatment or was added to it as the disease flared or progressed. Steroids and antibiotics are rarely given as monotherapy to prevent relapse in CD currently. Thus, studies that focus on their efficacy are commonly from the 1980s and 1990s. One such double-blind, randomized trial performed in 13 European centers followed 63 patients given budesonide and 66 patients given placebo post ileocelectomy. At 1 year, no difference in endoscopic recurrence was seen between

the 2 groups. However, a significantly lower endoscopic recurrence rate was seen in a subgroup of patients treated with budesonide, namely those who had undergone surgery for increased disease symptoms rather than obstruction or fistulization (32% *vs* 65% of the placebo group)^[64].

Studies on antibiotic monotherapy have been limited to metronidazole. In a double-blind controlled trial evaluating the use of metronidazole as monotherapy post ileal resection in 51 patients, recurrent lesions were

seen in 75% of the placebo group *vs* 52% of the metronidazole group at 12 wk ($P = 0.09$). At 1 year, clinical recurrence rates were much lower in the metronidazole group (4% *vs* 25%); however significance was lost at 2 years (26% *vs* 43%)^[65].

The commonly used aminosalicylate based drugs, including mesalamine, generally have low side effect profiles and have been shown by meta-analysis to prevent relapse in inactive CD^[66]. However, results from studies evaluating their long term efficacy in the prevention of post ileocelectomy recurrence are not impressive, particularly in regard to clinical recurrence. One large double blind, placebo controlled study by Sutherland *et al*^[67] compared clinical recurrence as defined by CDAI scores in medical and surgical CD patients (who had undergone a variety of resections) treated with either mesalamine or placebo. At 48 wk, 25% of the mesalamine *vs* 36% of the placebo had a clinical recurrence. However, disease recurrence was only 10 d later in the mesalamine treated patients. Interestingly, ileocolonic patients had fewer relapses on mesalamine (21% *vs* 41% given placebo) on subgroup analysis. In another double-blind, multicenter clinical trial published in 1995, 87 patients were treated with mesalamine or placebo within 1 mo after undergoing ileocelectomy. At 12 mo, 41% of the 17 patients who relapsed clinically had been given mesalamine. Using endoscopic and radiological evaluation with scoring systems, the mesalamine group had significantly less frequent and less severe lesions and milder disease. Disease was classified as "severe" in 24% of the mesalamine treated patients *vs* 56% of those given placebo^[68]. In another study published the same year, 163 post resection patients (of whom 109 had undergone ileocelectomy) were randomized to receive either mesalamine twice a day or placebo. During a maximum follow up of 72 mo, 31% of those given mesalamine experienced a symptomatic recurrence defined as symptoms plus endoscopic and/or radiological confirmation of disease *vs* 41% in the control group^[69].

A new paradigm of a "top down" approach to the treatment of CD in which surgery and early institution of immunomodulatory and/or biologic drug therapy has been suggested by large trials such as the SONIC trial. This approach has demonstrated improved mucosal healing, a reduction in steroid use, longer remission times and faster clinical response than the traditional bottom up approach^[70,71]. Additionally, multidrug therapy has been proven to increase drug serum levels, address multiple disease mechanisms and potentially reduce the production of anti-drug antibodies^[70,72,73]. The immunomodulatory drugs azathioprine (AZA) and 6 mercaptopurine (6 MP) are two drugs commonly used in the top down approach. Used on their own or in conjunction with other IBD medications, these drugs have a high success rate in treating flares, reducing the steroid requirement and increasing remission rates in medically treated disease and^[74,75] have been suggested to be effective at reducing recurrence and increasing duration from

surgery to recurrence. In a 5 center, double blind study inclusive of 131 post ileocelectomy patients randomized to receive 6 MP, mesalamine or placebo, only 6 MP was superior to placebo for the prevention of endoscopic and radiographic recurrence at the study endpoint of 24 mo. Clinical recurrence rates were improved by mesalamine or 6 MP administration with recurrence rates of 50% for 6 MP, 58% for mesalamine and 77% for placebo at 24 mo demonstrated. Endoscopic and radiologic recurrence rates were 43% and 33% for 6 MP, 63% and 46% for mesalamine and 64% and 49% for placebo^[76].

The utility of immunomodulatory drugs has been studied in subgroups of patients who are at increased risk for recurrence. D'Haens *et al*^[77] studied 62 patients who were aged < 30, had a history of multiple ileocelectomies and/or had penetrating disease. Post ileocelectomy, all patients were given metronidazole for 3 mo with either azathioprine or placebo for 12 mo. At 12 mo, a significant difference in endoscopic recurrence was observed with 43.7% of patients in the AZA group experiencing recurrence *vs* 69.0% of those in the placebo group. Endoscopically, 21% of the AZA group were lesion free *vs* 3% of the placebo ($P = 0.04$). Mañosa *et al*^[78] virtually reversed this study and administered AZA to 50 ileocelectomy patients postoperatively. At 3 mo, patients were randomized to receive either metronidazole or placebo. At 12 mo, endoscopic recurrence was seen in 36% of the metronidazole and 56% of the placebo group. However, this difference was not significant, suggesting that AZA on its own may be sufficient. In another high risk group, those undergoing their 2nd ileocelectomy for anastomotic recurrence, immunosuppressant drugs were shown to decrease clinical and surgical recurrence. Twenty-six patients were randomized to receive an immunosuppressant drug (6 MP, AZA, Methotrexate, $n = 14$) or a control treatment (5 ASAs, $n = 5$ or no treatment, $n = 7$). Clinical recurrence rates were lower in the immunosuppressant treated group *vs* the control group (3/12, 25% *vs* 6/10, 60%; $P < 0.05$). No difference in time to recurrence was demonstrated between the groups (approximately 27 mo in both groups). The control group required a 3rd resection more commonly (7/12, 58% *vs* 2/14, 17%, $P < 0.02$)^[79]. Other evidence suggests that AZA/6 MP treatment may delay but not prevent recurrence. Fifty-six consecutive patients commenced on AZA treatment immediately after resection were studied in Domènech *et al*^[80] observational study. Fifteen (27%) had ileocelectomies. Seventy percent of the cohort had endoscopic recurrence at 12 mo. However, no clinical recurrence was observed. At approximately 3 years' follow up, 30% of patients maintained endoscopic remission. At 5 years, the cumulative probability of endoscopic remission dropped to 18%. Due such evidence, the American Gastrological Association has recommended that as 6 MPs likely reduce the risk of clinical and endoscopic recurrence, they should be used in those at "high risk" for recurrence or 'in whom postoperative recurrence would have deleterious effects'^[81].

The anti-tumor necrosis factor (TNF) drugs, including infliximab and adalimumab are among the newest IBD drugs and have rapidly gained popularity over the past 10 years. A role for these drugs in the prolongation of postoperative remission has been suggested by preliminary studies. In one such study, significantly lower 1 year postoperative endoscopic and histologic recurrence rates were demonstrated in patients who had undergone ileal resection who were treated with infliximab. 9.1% (1 of 11) of these patients had endoscopic recurrence *vs* 27% (3 of 11) that were given placebo. Clinical remission rates were also higher in the infliximab group (80% *vs* 54% of the placebo group) but this difference wasn't significant^[82]. One prospective, multicenter but also small Italian study, aimed to determine if the administration of infliximab after diagnosis of postoperative endoscopic recurrence of ileocolic CD can induce endoscopic remission at 54 wk. Mesalamine was used as the control. In the mesalamine group ($n = 11$), no endoscopic remission was seen at 54 wk. Two patients had clinical recurrences at 8 and 9 mo. In the infliximab group ($n = 23$), 54% had endoscopic remission at 54 wk. None had clinical recurrence^[28].

Infliximab is the most commonly used and studied anti-TNF drug. However, a recent randomized control trial evaluated the efficacy of adalimumab for the prevention of post ileocelectomy recurrence^[83]. Fifty-one patients were randomized to receive adalimumab, AZA or mesalamine postoperatively. At 2 years, the adalimumab treated patients had the lowest incidence of endoscopic recurrence (6.3% *vs* 64.7% of the AZA group and 83.3% of the mesalamine group). Similarly clinical recurrence was lower in the adalimumab treated patients (12.5% *vs* 65% in both the AZA and mesalamine groups).

The timing of postoperative treatment has sparked a great interest due to the side effects of many IBD medications. The use of a "tailored treatment approach" to determine the effect of the timing of drug commencement on symptomatic recurrence after ileocelectomy was studied by Bordeianou *et al*^[14]. In their cohort of 199 ileocelectomy patients, 35% were given immediate post ileocelectomy prophylaxis in the form of antibiotics, 5 ASAs, immunomodulators (6 MP/AZA) and/or anti-TNFs. Sixteen percent were commenced on a drug regimen at the time of endoscopic recurrence and 49% percent did not receive any treatment. Symptomatic recurrence occurred in 29% of those treated immediately postoperatively *vs* 44% of those who were treated after recurrence. After multivariate analysis, the significant difference between the 2 groups was lost and the only remaining significant prognostic factor recurrence was Charlson Comorbidity Index^[84]. Malireddy *et al*^[17] studied pre *vs* postoperative administration of anti-TNFs and immunomodulators in 89 laparoscopic ileocelectomies. Timing of treatment did not affect recurrence rates. Postoperative medical treatment lengthened the time to recurrence with a median time to recurrence of 25 mo demonstrated in the group given pharmacoprophylaxis

vs 16 mo in the control group. However, this difference was not statistically significant.

SERUM MARKERS

Serum markers such as CRP and ESR are relatively non-invasive to obtain and have been demonstrated to reflect disease activity. Thus such markers may be potential prognosticators for post ileocelectomy recurrence. ESR and CRP were studied in a randomized controlled multicenter Italian trial of 98 patients undergoing their first ileocelectomy. When evaluating endoscopic recurrence at 6, 12, 24, and 36 mo post operatively, ESR and CRP were not correlated with endoscopic recurrence^[52].

Pro and anti-inflammatory cytokines are not measured in clinical practice. However, they offer the potential to be used as markers of disease activity and, possibly, disease recurrence. Yamamoto *et al*^[85] evaluated levels of the proinflammatory cytokines IL-6 (interleukin 6), IL1B and TNF α in blood, ileal biopsies and rectal biopsies at enrollment and 1 year after ileocelectomy in 36 patients. On univariate analysis, the 16 patients who experienced a clinical relapse (determined by CDAI scores) demonstrated significantly higher IL1B, IL-6 and TNF α levels in their ileal mucosa compared to the 20 patients who did not experience clinical relapse. There was no association with these markers in either the blood or rectal mucosa and relapse demonstrated. On multivariate analysis, IL-6 remained as an independent predictor of clinical relapse. IL-6 has also been demonstrated to be increased in the serum of CD patients with previously quiescent CD experiencing a disease flare^[86]. IL10 is a well-known anti-inflammatory cytokine. Meresse *et al*^[40] studied IL-10 levels and endoscopic recurrence in 36 patients 3 mo post ileocelectomy. Recurrence rate was 53%. Patients with recurrence had significantly lower IL-10 production. When ileal mRNA expression levels were compared with the patients' individual genotypes, varying IL10 production based on IL-10. G microsatellite genotype was seen. However, there was no correlation between genotype and endoscopic recurrence.

Faecal calprotectin (FC) and lactoferrin have been widely studied as noninvasive markers of gut inflammation. Recently, several groups have attempted to correlate levels of these markers with risk of postoperative recurrence in CD. Lamb *et al*^[87] were among the first to study a potential correlation. In their cohort of 13 post-surgical CD patients followed for 1 year (3 of whom had ileocolic disease) and a separate cohort of 104 patients who gave a single stool sample at a median of 24 mo postoperatively (28 of whom had ileocolic disease) both FC and lactoferrin correlated with clinical symptoms as evaluated by Harvey Bradshaw Index. Both markers were found to be more accurate at predicting clinical recurrence than CRP, platelet count and endoscopic appearance. Patients with Harvey Bradshaw scores indicative of severe disease activity ($n = 28$) had FC and lactoferrin levels of 661 $\mu\text{g/g}$ and 116.6 $\mu\text{g/g}$ *vs* 70.2 $\mu\text{g/g}$

and 5.9 µg/g in those with clinically inactive disease ($n = 43$) ($P < 0.001$). Although levels of both markers were slightly higher in those with endoscopic recurrence, this difference was not statistically significantly different between the groups. Subsequently, FC levels of 29 ileocelectomy patients were studied by Lobatón *et al.*^[88] Levels correlated more closely with clinical recurrence than CRP, white cell count and platelet count. Endoscopic recurrence scores also correlated with levels. Lasson *et al.*^[89] in a study published in Jan 2014, evaluated FC levels in 30 ileocelectomy patients in specimens collected monthly postoperatively for 1 year. Fifty-eight percent had endoscopic remission at one year. FC levels fluctuated over time and were mainly affected by diarrhea. Although median calprotectin levels were not significantly different between patients in remission and patients with recurrence, the majority of patients with high values had recurrence. This may have been influenced by diarrhea at the time of sampling.

CONCLUSION

Crohn's disease cannot be cured. Surgical resection offers an opportunity for "resetting" the disease into a state of temporary remission. It is known that after surgical resection recurrence rates range from 20 to over 60 percent. Identifying modifiable risk factors for postoperative disease recurrence can assist the clinician in implementing more aggressive prophylactic treatment to prevent recurrence and to sustain remission. The application of an optimal strategy for preventing postoperative recurrence is a multidisciplinary task that includes the gastroenterologist and the colorectal surgeon, as well as all the supporting staff that can ensure preoperative optimization, detailed and timely surgical intervention and early implementation of appropriate treatment to keep the patient in remission.

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Intestinal microbiota pathogenesis and fecal microbiota transplantation for inflammatory bowel disease

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Abstract

The intestinal microbiota plays an important role in inflammatory bowel disease (IBD). The pathogenesis of IBD involves inappropriate ongoing activation of the mucosal immune system driven by abnormal intestinal microbiota in genetically predisposed individuals. However, there are still no definitive microbial pathogens linked to the onset of IBD. The composition and function of the intestinal microbiota and their metabolites are indeed disturbed in IBD patients. The special alterations of gut microbiota associated with IBD remain to be evaluated. The microbial interactions and host-microbe immune interactions are still not clarified. Limitations of present probiotic products in IBD are mainly due to modest clinical efficacy, few available strains and no standardized administration. Fecal microbiota transplantation (FMT) may restore intestinal microbial ho-

meostasis, and preliminary data have shown the clinical efficacy of FMT on refractory IBD or IBD combined with *Clostridium difficile* infection. Additionally, synthetic microbiota transplantation with the defined composition of fecal microbiota is also a promising therapeutic approach for IBD. However, FMT-related barriers, including the mechanism of restoring gut microbiota, standardized donor screening, fecal material preparation and administration, and long-term safety should be resolved. The role of intestinal microbiota and FMT in IBD should be further investigated by metagenomic and metatranscriptomic analyses combined with germ-free/human flora-associated animals and chemostat gut models.

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Key words: Inflammatory bowel disease; Intestinal microbiota; Probiotics; Fecal microbiota transplantation; Synthetic microbiota transplantation

Core tip: Several lines of evidence strongly support the link between intestinal microbiota and inflammatory bowel disease (IBD). This review discusses the potential microbial pathogens, disturbance of intestinal microbiota, and immune interactions between host and microbes in IBD. Furthermore, alternative IBD treatment approaches aimed at restoring the disturbed intestinal microbiota have become a major interest in recent years. This article also reviews the present literature concerning the clinical use of probiotics, especially fecal microbiota transplantation and its barriers, and future directions in the management of IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic relapsing nonspecific inflammatory disorder of the gastrointestinal (GI) tract. The etiology of IBD is unknown. Contributing factors to the pathogenesis of IBD include disturbance of the intestinal microbiota and its metabolites, the host's genetic susceptibility, and the host's innate and acquired immunity^[1,2]. Many studies based on metagenomics have profiled the normal patterns of the human intestinal commensal microbiota. For example, 3.3 million microbial genes, up to 10 bacterial phyla, and > 1000 bacterial species (> 90% species belong to the phyla *Bacteroidetes* and *Firmicutes*) have been identified in the human intestine^[2,3]. Commensal fungi and viruses have also been detected in the human gut^[2,4]. Several lines of evidence strongly support the link between intestinal microbiota and IBD such as: (1) animal studies confirm that intestinal microbiota has an essential role in the pathogenesis of IBD, because colitis cannot be induced in germ-free animal models^[5,6]; (2) fecal stream diversion prevents recurrence of CD in the neoterminal ileum, but reinfusion of luminal contents into bypassed colonic segments rapidly results in recurrent disease^[7,8]; (3) fecal and intestinal mucosa-associated microbiota of IBD patients is characterized by decreased biodiversity and abnormal compositions (*e.g.*, an imbalance between protective and harmful microbes)^[2,9]; (4) the disturbed metabolites of intestinal microbiota (*e.g.*, abnormal butyrate metabolism) are implicated in the pathogenesis of IBD^[10,11]; (5) several probiotic products may be effective in relieving intestinal symptoms and preventing relapse in UC^[12]; (6) some non-absorbable antibiotics may induce and maintain remission in IBD^[12,13]; (7) many environmental factors such as the westernized diet, modern lifestyle, or abuse of antibiotics, have an important effect on the composition of intestinal microbiota and contribute to the significant increased incidence of IBD^[9,14]; and (8) IBD-related genetic susceptibility loci are mainly associated with the host-microbe immune interactions^[15-17].

Previously, researchers have tried to determine the specific microbial pathogens associated with the onset of intestinal inflammation of IBD^[18-22]. Recently, metagenomic studies have increased with the development of high-throughput DNA sequencing and bioinformatics analysis technology^[2,23,24]. Therefore, many studies have focused on the composition and function of gut microbiota in IBD patients, but the defined alteration of intestinal microbiota, the microbial interactions and host-microbe interactions are still not conclusive^[2,9,25]. Furthermore, IBD-related conventional medical treatments use aminosalicylates, steroids, immunosuppressive agents and biological therapies with many adverse effects, and no cure is available. Alternative IBD treatment approaches aimed at restoring the disturbed intestinal microbiota have become a major interest in recent years. Many clinical trials have been performed to investigate the efficacy of probiotics in IBD. The beneficial effect of

Table 1 Possible microbial pathogens associated with inflammatory bowel disease

<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i> ^[19,126,127]
Adherent-invasive <i>Escherichia coli</i> ^[118,44,45,128]
<i>Clostridium difficile</i> ^[107,129,130]
<i>Candida albicans</i> ^[21,49]
<i>Helicobacter</i> sp. ^[131-133]
<i>Campylobacter</i> sp., such as <i>Campylobacter jejuni</i> and <i>Campylobacter concisus</i> ^[134-136]
<i>Salmonella</i> sp. ^[135]
<i>Klebsiella</i> sp. ^[137]
<i>Yersinia</i> sp. ^[138]
<i>Listeria</i> sp. ^[139]
<i>Fusobacterium</i> sp. ^[140]
<i>Methanospaera stadmanae</i> ^[141]
<i>Bacteroides fragilis</i> ^[142]
<i>Norovirus</i> ^[143]

probiotics is modest, although several probiotic products can induce and maintain remission in UC^[26,27]. Recently, fecal microbiota transplantation (FMT) has re-emerged as a hot research topic^[28-30], largely due to its efficacy on the management of recurrent *Clostridium difficile* infection (CDI). FMT is now recommended as an alternative to standard therapy with antibiotics for recurrent CDI^[31,32]. Importantly, FMT may restore the balance of intestinal microbiota, so it is also proposed as an alternative treatment for IBD^[33]. Thus so far, several case series have shown the efficacy of FMT in refractory IBD, and IBD combined with CDI^[34-37], but it is not clear whether FMT has potential therapeutic value for IBD patients with mild IBD. FMT clinical application in IBD still leaves many unanswered questions. FMT-related screening of donor, fecal material preparation and administration is not standardized, and the defined microbial restoration mechanisms and long-term safety of FMT are still not clarified^[38,39]. Moreover, FMT researchers have to prepare and submit the complex investigational new drug applications in the future^[40]. This study reviews the present literature concerning the potential microbial pathogenesis in IBD, particularly FMT and its role in the management of IBD.

POTENTIAL MICROBIAL PATHOGENS IN IBD

Many studies have investigated the specific microbial pathogens contributing to the onset of IBD; however, no definitive pathogens have been confirmed^[10]. The potential bacterial, fungal or viral pathogens related to IBD are listed in Table 1. *Mycobacterium avium* subspecies *paratuberculosis* can colonize the ileal mucosa of CD patients^[19], which has been often linked to the etiology of CD, but with no conclusive evidence to its involvement^[41-43]. Moreover, *Escherichia coli* (*E. coli*) strain LF82 was isolated from the ileum of CD patients^[18]. Several studies have shown that CD patients have a higher prevalence of adherent-invasive *E. coli* (AIEC) in ileal lesions, which indicates a specific association of AIEC with CD^[20,44]; higher expression of the outer membrane porin C of

AIEC can be observed in patients with CD^[45]. AIEC proliferation has also been found in the colonic mucosa of UC patients^[46]. Although the enhanced adherence and invasion of AIEC is present among IBD patients, the potential mechanisms between AIEC and IBD still need to be clarified. Furthermore, CDI is common among IBD patients, and similar symptoms between CDI and IBD makes it difficult to distinguish between them^[47]. CDI can activate the intestinal proinflammatory response and is responsible for the development or exacerbation of IBD. IBD itself may contribute to the increased risk of CDI^[47]. However, there is no clear evidence that CDI precedes IBD. Much evidence has shown that fungal pathogens may be involved in the pathogenesis of IBD, especially CD^[1]. Anti-*Saccharomyces cerevisiae* antibodies (ASCAs) as one of the serological markers for CD can also be induced by *Candida albicans* (*C. albicans*)^[7,48]. *C. albicans* can be isolated from the intestine more frequently in CD patients and their healthy relatives, but the positive association between ASCAs level and the amount of *C. albicans* in CD is still controversial^[49,50]. Inhibition of interleukin (IL)-17A by secukinumab is ineffective in active CD patients^[51], which may be linked to *C. albicans* thriving in the gut induced by loss of control by IL-17^[52]. In addition, large amounts of *Candida* sp. can also be detected in the feces or intestinal mucosa among UC patients^[50], and the clinical symptoms and intestinal inflammation may be improved after antifungal treatment. Although many studies have shown a higher prevalence of pathogenic microbes in IBD, no specific pathogenic microbe has been identified to date, and the cause and consequence relationship of the single pathogenic microbe and IBD development is still controversial. Increasing evidence has confirmed that the disturbance of the intestinal microbial community may be responsible for the pathogenesis of IBD.

DISTURBANCE OF INTESTINAL MICROBIOTA IN IBD

With the development of culture-independent techniques such as metagenomic analysis, the disturbance of intestinal microbiota associated with IBD has been better described. This includes the involvement of the feces/colonic mucosa-associated microbiota, inflamed lesions-/normal mucosa-related microbiota^[53], and even the intestinal microbiota in IBD remission and relapse^[54]. Although the conclusions about the altered intestinal microbiota are still uncertain, more consistently observed alterations of intestinal bacterial microbiota linked to IBD have been reported. The decrease in biodiversity and depletion of the phyla *Bacteroidetes* and *Firmicutes* can be observed in feces/mucosa-associated microbiota among IBD patients^[2,55,56]. The bacterial communities in the intestine are significantly different between UC and CD patients and healthy individuals^[57,58]. Furthermore, at the genus level, many potentially protective bacteria and normal anaerobic bacteria such as *Bacteroides* sp., *Eubacterium*

sp. and *Lactobacillus* sp., are significantly decreased both in active UC and CD patients, and even in patients with inactive IBD^[59-68]. In addition, the abnormal metabolites of intestinal bacterial microbiota can also contribute to the pathogenesis of IBD^[69,70]. For example, butyric acid, which is the main energy source of the intestinal epithelial cells, can inhibit the signal pathway of proinflammatory cytokines. Butyrate-producing bacteria and their culture supernatants can improve the intestinal inflammation and necrosis in the animal model of colitis^[71]. In parallel, some studies have confirmed that the levels of some butyrate-producing bacteria (e.g., *Clostridium* clusters IV and XIVa) and the availability of butyrate reduced significantly in UC patients^[11,72]. Moreover, the metabolic activity of intestinal microbiota in UC is disturbed, with increased levels of taurine and cadaverine^[73]. Until now, the highly reproducible profiles of intestinal microbiota established for IBD patients have been limited. Recently, one study based on a phylogenetic network analysis showed that the human intestinal mucosal bacterial community could be organized into five preserved microbial modules and two IBD-associated microbial modules displayed enhancement of the oxidative response and glycan metabolism pathways relevant to host-pathogen interactions^[25].

The fungal communities are also important components of microbiota in the human GI tract; most of which have co-evolved with the host in a symbiotic relationship^[21]. Early studies based on culture-dependent methods reported that fungi were detected in the digestive tract of 70% of healthy adults^[74], and the number of fungi in the human colon is 10²-10⁶ cfu/mL; most of which are aerobic or facultatively anaerobic. Recently, metagenomic analysis of 124 individuals reported that only 0.1% of microbial genes in feces were of eukaryotic or viral origin^[2], which was consistent with previous reports of intestinal microbiome accounting for 0.03% of the fecal microbiota^[75]. There is limited information available about the prevalence and classification of the intestinal fungal microbiota. The study of the fungal microbiota is in its infancy, and much remains to be determined^[75]. There were significant differences in fungal communities related to IBD compared to non-IBD controls. The fungal sequences could be detected in the colonic mucosa of all IBD patients, and the diversity of the intestinal microbiome increased clearly among IBD patients, but the proportion of microbiome in the whole intestinal microbiota was low^[75]. Moreover, whether the intestinal microbiome interacts with the mucosal immune system or influences intestinal disorders is unknown. Recently, a study connected intestinal fungal microbiota with the host immune system through Dectin-1 in a mice model of dextran-sulfate-sodium-induced colitis^[1], which confirmed the fungal etiology in IBD.

Overall, the diversity and abundance of intestinal bacterial microbiota are reduced in IBD, and the bacterial microbiota metabolites are also disturbed. However, a specific IBD microbiota has not yet been revealed, which might in part be because of inter-individual vari-

ability, different IBD characteristics or subtypes, and different data analysis methods. In addition, whether the microbiota pathogenesis is the initiating factor in IBD or is secondary to IBD still cannot be answered. Moreover, further studies based on fungal high-throughput DNA sequencing should be conducted on whether the change of the fungal community structure is secondary to the imbalance of the intestinal bacterial community, or independent pathogenic factors of IBD.

HOST-MICROBE IMMUNE INTERACTIONS IN IBD

The human immune system is usually tolerant of the commensal microbiota colonized in the GI tract. Abnormal activation of the host immune response against the imbalanced intestinal microbiota may be the potential pathogenesis mechanism of IBD^[76]. Additionally, an abnormal intestinal microbiota offers persistent stimulation of the immune system in individuals who are genetically susceptible to IBD, which results in dysfunction in immune tolerance and regulation. The resulting chronic intestinal inflammation in the GI tract can initiate intestinal lesions and lead to IBD-associated symptoms. Intestinal mucosal epithelial cells are continuously exposed to the intestinal microbiota, and they can recognize various cell wall components of pathogenic and commensal microbes, which are the main sources of pathogen-associated molecular patterns. The pattern recognition receptors (PRRs) on intestinal cells include Toll-like receptors (TLRs)^[77], NOD-like receptors (NLRs) and C-type lectin receptors (CLRs), which are essential for a human host to recognize endogenous/exogenous microbes, and trigger and maintain intestinal mucosal innate and acquired immunity. The immune responses mediated by PRRs on intestinal cells include microbial binding and phagocytosis; induction of antimicrobial effect or mechanisms; and the production of endogenous antimicrobial peptides, cytokines and chemokines. Moreover, the majority of IBD-related genetic susceptibility loci are associated with PRRs. For example, the polymorphisms in NOD2 have been identified to increase the risk of CD in western populations, and the polymorphisms in TLRs are associated with UC and CD. In addition, CLRs such as Dec-1 can contribute to the recognition of intestinal fungi and influence the immune function of intestinal mucosa in UC^[1].

CLINICAL USE OF PROBIOTICS IN IBD

The intestinal dysbiosis in IBD has been confirmed by molecular techniques, and there is a compelling rationale for modulating the altered intestinal microbiota among IBD patients. Animal studies have confirmed that probiotics enhance the intestinal mucosal barrier function, regulate intestinal mucosal immunity, recover intestinal microbial community structure, and improve chronic intestinal inflammation. Clinical trials in humans have in-

vestigated whether the present probiotic products could be a treatment option in IBD. Some bacterial strains of *Lactobacillus* sp.^[78,79], *Bifidobacterium* sp.^[80,81], *Escherichia* sp. (e.g., *E. coli* Nissle 1917)^[82,83], and the fungal strain *Saccharomyces boulardii* (*S. boulardii*)^[84,85], are the most common investigated probiotics in the treatment of IBD. In addition, except for the single probiotic strain, some probiotic combinations such as VSL#3, an eight probiotic consisting of four strains of *Lactobacillus*, three strains of *Bifidobacterium*, and one strain of *Streptococcus*, have shown efficacy by maintenance of remission both in adults and children with active IBD^[86-88].

According to the efficacy of probiotics in UC, patients may experience fewer relapses when probiotics of *Lactobacillus* sp. are added to their usual therapies or when they cannot tolerate standard medications^[89]. In addition, *E. coli* Nissle is an effective alternative to aminosalicylates for maintenance of UC remission^[82,83]. Administration of *S. boulardii* during maintenance treatment with mesalazine induced clinical remission in 71% of patients with active mild to moderate UC^[85]. Moreover, VSL#3 resulted in a combined induction of remission/response rate of 77% among patients with active mild to moderate UC and who did not respond to conventional therapy^[87]. Furthermore, the effects of probiotics on pouchitis are by far the most convincing data. VSL#3 is more effective than placebo for prevention and treatment of pouchitis for postoperative UC patients^[90]. Overall, a Cochrane review showed that conventional therapy combined with probiotics has few beneficial effects on the induction of remission in active mild to moderate UC, but probiotics may be useful in the maintenance of remission in non-active UC and in the prevention of postoperative recurrence; the outcomes from the present clinical trials on probiotics in UC need to be confirmed. On the other hand, current data show that *S. boulardii* has no beneficial effects on maintaining remission in CD patients^[84], but might lead to fewer relapses when combined with mesalamine^[91]. Moreover, probiotics of *Lactobacillus* sp. cannot prevent postoperative relapse of CD^[78,92]. There is insufficient evidence to draw any conclusions about the efficacy of probiotics for induction or maintenance of CD remission^[27]. Importantly, *Faecalibacterium prausnitzii* (*F. prausnitzii*) identified as a butyrate-producing species, may contribute to the gut homeostasis and play a protective role in IBD, especially in CD^[71]. The levels of *F. prausnitzii* in feces and intestinal mucosal biopsies decrease both in CD and UC^[93-96], and the lower level of *F. prausnitzii* on the ileal mucosa of CD patients is associated with recurrence. However, one study showed an increased level of *F. prausnitzii* in mucosal biopsies associated with reduced bacterial diversity in pediatric CD^[97]. Generally, *F. prausnitzii* is a potential and promising probiotic, and its protective role in IBD is worthy of investigation.

In summary, the clinical efficacy of the present probiotic products in the treatment of IBD is modest; they are currently used only as supplements in IBD treatment, and not as alternatives or substitutes for conventional

therapy. The application of probiotics should be based on the principles of evidence-based medicine, but well-designed randomized controlled trials (RCTs) are lacking. Further appropriate study designs and larger numbers of patients will be needed to determine the optimal probiotics for IBD.

INDICATIONS FOR FMT

FMT, also called stool/fecal transplantation or fecal bacteriotherapy, refers to infusion or engraftment of a homogenized fecal suspension from a healthy individual into the GI tract to cure a specific disease^[29,32,98]. Due to the elucidation of the composition and function of intestinal microbiota by the development of metagenomics studies, many researchers have begun to explore therapeutic interventions in human diseases associated with intestinal dysbiosis from the viewpoint of microecology. Although various probiotics, prebiotics and synbiotics have been administered in clinical practice, most are recommended as supplementary treatments due to poor therapeutic effects and the limited number of available strains. So far, only the preliminary application of FMT exhibits marked clinical effectiveness, especially in the treatment of recurrent CDI and IBD^[30,33,99,100]. Traditionally, CDI is mainly treated with antibiotics such as vancomycin and metronidazole. However, the therapeutic effect is poor due to the drug resistance of *C. difficile*. The recurrence rate of CDI is much higher, and can be up to 15%-26%^[101]. FMT can be considered to replace antibiotics for recurrent and refractory CDI that has relapsed more than three times^[31]. A recent RCT about FMT suggested that 81% of recurrent CDI patients achieved remission of symptoms after FMT, while only 31% of patients receiving only vancomycin treatment exhibited symptom remission^[102]. A multicenter long-term follow-up study on FMT treatment of recurrent CDI through a colonoscopy route demonstrated a 91% primary cure rate and 98% secondary cure rate^[101]. A recent systematic review showed that FMT could achieve clinical remission in 63% of IBD patients, while 76% of the patients could stop taking IBD-related drugs and their GI symptoms were reduced^[33]. Several preliminary studies using FMT for GI disorders including irritable bowel syndrome, antibiotic-associated diarrhea and chronic constipation have also met with some success. In addition, non-GI disorders such as diabetes mellitus and insulin resistance^[103], metabolic syndrome, childhood autism, chronic fatigue syndrome, multiple sclerosis, fibromyalgia, myoclonus dystonia and Parkinson's disease are reported to be improved and cured with FMT^[39,104].

FMT IN THE MANAGEMENT OF IBD

The treatment of IBD is rapidly evolving, and many conventional and novel drug treatments have proven effective, including aminosalicylates, steroids, immunosuppressive agents and biological therapies. However, some

patients become refractory to standard management, and some have significant adverse effects, with many patients requiring surgery. Despite medical treatment, a significant number of patients live with mild active symptoms and have a poor quality of life. Given the role of the intestinal microbiota in driving inflammation in IBD, treatments that manipulate the microbiota have been investigated including the use of probiotics and prebiotics, with variable evidence for their efficacy. FMT is becoming an alternative microbiota treatment for IBD with astounding efficacy.

The main case series and case reports of FMT for IBD treatment are shown in Table 2. The first case report of FMT for IBD was published in 1989, in which the author himself confirmed UC for 7 years that was refractory to sulfasalazine and steroids. Six months after transplantation of a healthy donor stool by retention enemas, he remained symptom free^[34]. Moreover, a case series of six patients with refractory UC apparently achieved complete, medication-free remission after FMT with no disease recurrence after 1-13 years follow-up^[35]. Recently, a system evaluation reported that FMT could achieve clinical remission in 63% of IBD patients, while 76% of patients could stop taking IBD-related drugs and their GI symptoms were reduced^[33]. However, a study including five patients with moderate to severe active UC showed that none of them achieved remission after FMT by week 12, and a positive clinical response was observed only in one patient^[105]. The poor response in that study may have been associated with the severity of UC itself, rather than the optimal administration of FMT. FMT may be an optimal treatment for refractory IBD with no response to current conventional therapy, such as anti-inflammatory agents, steroids, immunosuppressive and biological drugs. For refractory IBD, continuously repeated FMT is needed to cure or achieve effective remission^[38]. However, no unified standard exists in the procedures of FMT treatment in IBD. In addition, CDI is common among IBD patients with an incidence of 3.7%^[106]. Once combined with CDI, the severity of IBD can be aggravated, while the recurrence rate of CDI can be increased^[107]. There is no standard therapy for UC combined with CDI. For example, vancomycin and metronidazole would be preferred but with poor efficacy, and it remains controversial whether IBD-related therapeutics should be continuously used. FMT may be a viable therapeutic approach for IBD combined with CDI. Recently, one study evaluated the feasibility and safety of FMT in 10 children with UC. After FMT by retention enemas (freshly prepared fecal enemas) daily for 5 d, 78% and 67% of patients achieved a clinical response within 1 wk and 1 mo, respectively^[108]. Overall, studies of FMT in IBD are rare and restricted to case series or reports, so the available evidence is limited and weak. However, FMT still has the potential to be an effective and safe treatment when standard IBD management has failed. Compared with CD, the efficacy of FMT in UC is more promising, but further investigation is required^[33,39,109]. Moreover, except for refractory IBD, it is

Table 2 Main case series and reports of fecal microbiota transplantation in inflammatory bowel disease treatment

Ref.	IBD type (n)	Stool material	Volume infusion	Infusion route	Frequency	Donor relationship	Characteristics of outcomes
Bennet <i>et al</i> ^[134] , 1989	UC (1)	NR	NR	Enema	1	NR	Documents remission for 6 mo and cease medications
Borody <i>et al</i> ^[144] , 1989	UC (1)	NR	NR	Enema	NR	NR	Documented remission for 3 mo and cease medications.
Borody <i>et al</i> ^[35] , 2003	UC (6)	Fresh	200-300 g/ 200-300 mL	Enema	6	Related or unrelated	Documented remission from 1 to 13 yr and cease medications
Hamilton <i>et al</i> ^[112] , 2012	UC combined with CDI (4)	Fresh or frozen	220-240 mL	Colonoscopy	1	Related or unrelated	Colitis activity was improved, and CDI was cured
Zainah <i>et al</i> ^[145] , 2012	UC combined with CDI (1)	Fresh	300 mL	Colonoscopy	1	Related	Documented symptom-free for 8 mo without CDI recurrence
Borody <i>et al</i> ^[146] , 2012	UC (3)	Fresh	NR	Repeated rectal infusions	Daily infusion for 2 to 6.5 mo	Related or unrelated	Documented improvement from 1 to 36 mo
Patel <i>et al</i> ^[147] , 2013	UC combined with CDI (3)	Fresh	18-397 g/ 180-600 mL	Colonoscopy	1	Related or unrelated	Symptoms such as diarrhea improved or resolved 3 mo after FMT
Angelberger <i>et al</i> ^[105] , 2013	UC (5)	Fresh	17-25 g/250 mL + 6-12 g/100 mL	Nasojejunal tube + enema	3	Unrelated	None of cases achieved remission, but only one case was response to FMT by week 12; two cases deteriorated 4 wk after FMT
Kump <i>et al</i> ^[148] , 2013	UC (6)	Fresh	300-500 mL	Colonoscopy	1	Unrelated	Documented improvement, but no remission within 2 wk after FMT
De Leon <i>et al</i> ^[110] , 2013	UC combined with CDI (1)	Fresh	600 mL	Colonoscopy	1	Related	UC relapse 9 d after FMT
Kunde <i>et al</i> ^[108] , 2013	UC (10)	Fresh	165 ml	Enema	5	Related	78% and 67% subjects achieved clinical response within 1 wk and 1 mo after FMT, respectively
Borody <i>et al</i> ^[144] , 1989	CD (1)	NR	NR	Enema	NR	NR	Symptoms-free and receiving no medications 4 mo after FMT
Grehan <i>et al</i> ^[118] , 2010	CD (1)	Fresh	200-400 mL	Colonoscopy + enema	1 + 9	NR	CD related improvement was not reported
Hamilton <i>et al</i> ^[112] , 2012	CD combined with CDI (6)	Fresh or frozen	220-240 mL	Colonoscopy	1 or 2	Related or unrelated	Two cases accepted the second FMT due to CDI recurrence, but the efficacy of FMT on CD was not reported
Patel <i>et al</i> ^[147] , 2013	CD combined with CDI (2)	Fresh	18-397 g/ 180-600 mL	Colonoscopy Upper endoscopy	2	Related or unrelated	CDI recurred in 1 case after the first FMT by colonoscopy, and a second FMT was performed by upper endoscopy; but the efficacy of FMT on CD was not reported
Gordon <i>et al</i> ^[109] , 2013	CD (1)	Fresh	NR	NR	NR	Related	Response to FMT for 6 mo and then relapsed
Quera <i>et al</i> ^[149] , 2013	CD combined with CDI (1)	NR	NR	Colonoscopy	NR	NR	Transient bacteremia occurred 24 h after FMT Documented symptom-free 5 mo after FMT and CDI disappeared
Zhang <i>et al</i> ^[36] , 2013	CD (1)	Fresh	150 mL	Gastroscope	1	Related	Documented clinical remission for more than 9 mo

FMT: Fecal microbiota transplantation; UC: Ulcerative colitis; CDI: *Clostridium difficile* infection; CD: Crohn' disease; NR: Not reported.

not yet clear whether FMT has any potential therapeutic value for IBD patients induced into remission *via* conventional medical therapy or those with mild IBD.

With regard to adverse events of FMT in IBD treatment, some patients may exhibit belching, abdominal distension, abdominal colic, diarrhea, constipation and other short-term symptoms. Fever and a temporary increase of C-reactive protein can develop transiently after FMT^[105]. However, most of these discomforts and symptoms disappear within 2 d after transplantation^[37]. Furthermore, other rare complications such as GI bleeding and peritonitis are mainly related to the endoscopic procedures in the process of FMT. Few serious adverse events occur during treatment of IBD by FMT, but it is noteworthy that IBD-related symptoms can be aggravated by FMT in

some cases with moderate to severe UC^[105]. Furthermore, one case report showed that FMT caused UC-related intestinal inflammation in one elderly male patient with CDI, who had been in long-term remission of UC for > 20 years without any UC-related treatment^[110]. Moreover, the long-term follow-up data of FMT such as infection, intestinal inflammation and tumors are still lacking, and need to be further investigated. Nevertheless, there are many impediments limiting the therapeutic potential of FMT in IBD^[111], such as rare FMT trials in IBD, ethical and social issues, poor screening of donors, no standard administration of FMT, no standardized preoperative preparation and pretreatment, no standardized preparation of fecal samples, and a lack of FMT-related basic investigations. All the above-mentioned limitations will

Table 3 Donor selection for fecal microbiota transplantation

Absolute exclusion criteria ^[32,104,124]
Failed to provide informed consent
Systematic and local microbial infections (<i>e.g.</i> , pathogenic bacteria, virus, ova and parasites)
Current communicable diseases
Malignancy and chemotherapeutics administration
Chronic gastrointestinal disorders
Peptic ulcer diseases
Gastroesophageal reflux disease
GI polyposis
Inflammatory bowel disease
Irritable bowel syndrome
Chronic constipation
Traveler's diarrhea
Current GI symptoms
Antibiotics administration
Immunosuppressive agents and biological agents
Other medications impact on the gut microbiota (<i>e.g.</i> , proton pump inhibitor, prokinetic agents, steroids, aspirin, probiotics, <i>etc.</i>)
High-risk lifestyles (<i>e.g.</i> , intravenous drug abuse, risk sexual behaviors, <i>etc.</i>)
Relative exclusion criteria ^[32,104,124]
Age < 18 and > 70 yr
History of major GI surgery
Metabolic syndrome
Diabetes mellitus
Abnormal body mass index (< or > 18-25 kg/m ²)
Systemic autoimmune disease
Atopic diseases(<i>e.g.</i> , asthma and eczema)
Chronic pain syndromes (<i>e.g.</i> , chronic fatigue syndrome and fibromyalgia)
Neuropsychiatric diseases

be discussed in the following sections.

BARRIERS AND FUTURE DIRECTIONS OF FMT CLINICAL PRACTICE IN IBD

Screening and selecting criteria for donors

Microbiota donation has a higher requirement for the screening of donors in the management of patients undergoing FMT, compared with blood donation. The donor must provide informed consent and detailed medical certificates including medical history, relevant examinations, stool and serological testing. Currently, the donor's inclusion and exclusion criteria are mainly from the self-determined standards of different studies and are more consistent overall, and are listed in Table 3. The feces and serological screening for common and known microbial pathogens are shown in Table 4. The donors are mainly selected from individuals who are closely related to the recipient including intimate partners, family members, and friends, whose microecological environment may be similar to that of the recipient; therefore, a more positive outcome to FMT may be produced, at least theoretically. Most opinions are that the stools from relatives or friends of the recipients show better efficacy compared with those from unrelated donors, and the difference in sex between donor and recipient had little impact on disease remission, but this conclusion is still lacking evidence^[99]. Unrelated healthy individuals are also potential donors.

Table 4 Donor screening for fecal microbiota transplantation

Common and entail serologic screening items ^[32,124]
Blood routine
Blood biochemistry
Human immunodeficiency virus-1 and -2
Hepatitis A, B and C virus
Syphilis
<i>Helicobacter pylori</i>
Human T lymphotropic virus
Cytomegalovirus
Epstein-barr virus
Common and entail stool screening items ^[32,124]
Stool routine
<i>Clostridium difficile</i> toxin A/B
<i>Salmonella</i> sp.
<i>Shigella</i> sp.
<i>Campylobacter</i> sp.
<i>Escherichia coli</i> O157
<i>Staphylococcus aureus</i>
<i>Yersinia</i>
<i>Helicobacter pylori</i>
<i>Vibrio parahaemolyticus</i> and <i>Vibrio cholerae</i>
<i>Candida albicans</i>
Rotavirus
<i>Cryptosporidium</i>
<i>Giardia</i>
<i>Cyclospora</i>
<i>Isospora</i>
Ova and parasites

Donor feces can be frozen and thawed without loss of effectiveness, enabling FMT-related microbiota banking^[112]. In addition, the human intestinal microbiota can be divided into three types: *Bacteroides*, *Prevotella* and *Ruminococcus* by the high-throughput sequencing methods^[24]. This means that matching enterotype between donor and recipient based on metagenomic analysis may improve the therapeutic efficacy of FMT. At present, FMT from a healthy donor is mainly used to perform allogeneic FMT. The fecal samples are mainly obtained from a related or unrelated healthy donor, who must face the series of ethical issues in receiving another person's feces, and the effects of the donor's intestinal microbiota on the intestinal immune and pathophysiological functions are unclear without effective theoretical supports. For those patients with mild IBD, it may be good that the fecal samples can be collected and stored in the remission stage and offered to the same patients when they come into the active stage of IBD. However, whether autologous or allogeneic FMT can relieve IBD-related clinical symptoms and induce/maintain the remission of IBD effectively is worthy of further investigation.

Preoperative preparation of donor and recipient

At present, a standardized method of IBD-related preoperative preparation for FMT has not been established^[52]. The donor should be administered a gentle osmotic laxative the night before FMT, and should avoid any infections between screening and time of donation. For the FMT recipient, large-volume bowel preparation (*e.g.*, polyethylene glycol) is required regardless of the route of

FMT. In addition, GI motility inhibitors such as loperamide may be optional for the retention of transplanted microbiota, and proton pump inhibitors should be given to recipients before FMT *via* upper GI routes. Importantly, current studies claim that IBD patients who intend to receive FMT need to receive antibiotic pretreatment^[35,105], although it still needs to be verified whether antibiotic pretreatment is necessary. In animal models, antibiotic pretreatment before FMT can cause serious damage to the intestinal microbiota structure and may affect the intestinal colonization of the donor's microbiota^[113].

Preparation of fecal samples

Generally, the preparation of fecal samples for IBD treatment can refer to the FMT guidelines for treating CDI^[32]. FMT practitioners face many challenges in implementing protocols for donor fecal preparation in a clinic setting. Donor fecal samples should be kept in an airtight container and chilled. The samples should be delivered to the institution preferably within 6 h of passage to undergo dilution with proper diluents such as normal saline, homogenization with a blender to achieve a liquid slurry, and filtration to remove particulate matter^[39]. Several issues should be noted during the fecal preparation. Initially, the majority of studies utilized fresh fecal material, which means that the fecal collection, preparation and transplantation should be performed on the day of planned FMT^[102]. However, fresh fecal material for FMT is not always practical due to issues including delay caused by screening tests, sanitation and aesthetics. Several studies have confirmed that frozen donor fecal material has equal efficacy in the treatment of recurrent CDI compared with fresh fecal material^[112]. The frozen material preparation should be processed within 2 h of collection; the specific steps of which are in accord with those of fresh fecal material; the finished fecal suspensions should be stored at -80 °C after adding sterile glycerol. On the day of FMT, the frozen material is thawed and diluted with sterile normal saline^[112]. Importantly, the frozen preparation is beneficial to establish the FMT-related gut microbiota banks.

In addition, the ideal volume of fecal material for instillation has not been standardized. A systematic review showed that > 500 mL of fecal suspensions allowed 97% of patients with recurrent CDI to achieve remission, while only 80% of patients obtained remission with 200 mL of fecal suspensions^[99]. However, it is difficult to standardize the procedure due to the different diluted concentrations of fecal material. Use of < 50 g fecal material may increase the relapse rate of CDI by four times compared with > 50 g fecal material^[99]. Overall, practitioners who regularly perform FMT favor 50-60 g of 250-300 mL diluent, respectively^[104]. Larger volumes (*e.g.*, 250-500 mL) should be used for delivery from the lower GI tract (*e.g.*, *via* colonoscopy or enema), and smaller volumes (*e.g.*, 25-50 mL) should be used for delivery from the upper GI tract (*e.g.*, *via* a nasoenteric or nasogastric tube). FMT practitioners prefer normal saline, sterile water or 4% milk

to dilute the stool sample at present, but which is the optimal diluent still needs to be investigated^[99].

Route of FMT administration

FMT is mainly performed *via* the lower GI route, including colonoscopy and retention enema, and/or *via* the upper GI route such as nasoenteric tube, nasogastric tube and gastroduodenoscopy. To date, approximately 75% of cases with recurrent CDI worldwide are administered with FMT *via* the lower GI tract and 25% *via* the upper GI route^[104]. FMT *via* the lower GI tract may be more effective than *via* the upper GI tract, although this has yet to be validated^[99,100]. Until 1989, retention enema was the most common technique for FMT; however, various alternative methods including nasoenteric tube, gastroscopy and colonoscopy have been used subsequently. A recent long-term follow-up study that involved 77 patients with recurrent CDI showed that colonoscopic FMT was well received by participants and was highly successful, with an overall primary cure rate of 91% and a secondary cure rate of 98%^[101]. Moreover, a systematic review showed that colonoscopic FMT had a higher cure rate (91%) for recurrent CDI, compared with the other upper GI routes^[99]. Colonoscopic FMT has even been proposed as first-line therapy for the treatment of CDI^[114]. For example, the fecal suspensions are sprayed through the biopsy channel of colonoscopy from the terminal ileum, while the colonoscopy is slowly removed until approximately 500 mL of fecal suspensions thoroughly perfuse the colon. Patients should avoid defecation within 1 h after FMT. In patients with severe colitis and significant colonic distention, colonoscopy may be technically challenging and potentially dangerous. Until now, few studies have directly compared routes of FMT administration.

A recent RCT demonstrated that 81% of patients had resolution of recurrent CDI after the first FMT by duodenal infusion^[102]; the remarkable cure rate by the nasoenteric tube matched that of colonoscopic FMT. FMT through the upper digestive tract is easy to perform and has a low risk, but it remains unclear whether donor fecal material can be distributed throughout the full colon and increase the risk of small intestinal bacterial overgrowth. According to the optimal FMT route for IBD, a study that involved IBD cases worldwide showed that up to 80% of IBD patients were administered FMT by colonoscopy and/or retention enema^[33]. Overall, there are many unanswered questions regarding the best route of administering FMT; the standardized and optimal route for FMT is determined by the needs and status of the patients, and the intestinal microbiota characteristics^[103].

Potential therapeutic mechanisms of FMT

Theoretically, the fecal suspension from a healthy donor can reconstruct the damaged intestinal microbiota, restore the intestinal colonization resistance and defend against colonization and infection with *C. difficile* and other pathogenic microorganisms. Thus, the imbalanced structure and function of the intestinal flora is restored

to improve the relevant clinical symptoms. However, the beneficial changes in the intestinal flora generated by FMT and its potential mechanisms are still not clear. Recently, high-throughput sequencing and bioinformatics techniques have been widely applied to describe the intestinal microbial community structure and functions. So far, only a few studies have been conducted that aim to analyze the restoring mechanisms of FMT on the recipient's damaged intestinal microbiota. Several studies based on the bacterial 16S rDNA-based sequencing analysis in recurrent CDI patients found that the diversity and richness of fecal microbiota was clearly reduced^[115-117]. The significant changes in *Clostridiales* and *Lactobacillales* from the phylum *Firmicutes*, and *Enterobacteriales* from the phylum *Proteobacteria* could be observed between recurrent CDI and post-FMT patients and healthy donors^[116]. The fecal microbiota of recurrent CDI patients becomes more similar to that of healthy donors after FMT, which means that FMT has a permanent action in improving the damaged intestinal microbiota and the clinical symptoms of the recipient^[116,117]. Moreover, the reconstructive efficacy of FMT can be maintained for a long time and last for 24 wk^[118]. Recently, one study based on the 16S rDNA pyrosequencing showed that abundant donor-related bacterial microbiota could be established in UC recipients, but the efficiency and stability of donor microbiota colonization varied greatly^[105]. Several anti-inflammatory and/or short-chain-fatty-acid-producing species such as *F. prausnitzii*, *Roseburia faecis* and *Bacteroides ovatus* were only able to colonize successfully in one UC patient with a positive clinical response after FMT for up to 12 wk^[105]. Overall, the influence of FMT on the structure/stability of the intestinal microbiota in IBD patients remains unclear. More longitudinal human and animal studies are needed to verify the permanent reshaping mechanism of FMT. At present, metagenomic analysis combined with germ-free animals, human flora-associated animals, and the chemostat gut models *in vitro* can be used to investigate the mechanism of FMT-related restoring of intestinal microbiota.

An investigational new drug application for FMT

Recently, the US Food and Drug Administration (FDA) has tightened the regulations of FMT, because the complex nature of the fecal microbiota products present specific scientific and regulatory challenges. FDA has announced that fecal microbiota collected from healthy individuals is a biologic product, meaning physicians must submit an investigational new drug (IND) application. However, published data have confirmed the astounding efficacy of FMT, which may be the best therapeutic modality for the treatment of recurrent CDI.

Physicians and scientists are concerned that it is no easy task to file the IND application for FMT practitioners, which could make FMT unavailable and suggest an alternative regulatory approach to ensure the widespread availability of FMT for those patients with recurrent CDI. FDA has acknowledged these concerns and has

published the alternative enforcement discretion regarding the IND requirements for the use of FMT to treat CDI not responding to standard therapies. FDA intends to exercise this discretion provided that the FMT practitioners obtain adequate informed consent for the use of FMT products, stating that FMT products to treat CDI are investigational drugs, and explaining any potential risks for FMT treatment. Unfortunately, the use and study of FMT for diseases or conditions other than CDI is not included in this enforcement discretion policy. Therefore, physicians wishing to ensure access to FMT for IBD or other conditions need to file an IND application. Recently, several articles provided step-by-step guidance to physicians on how to navigate the regulatory requirements of FDA and prepare the IND application for FMT^[40,119]. The use of an IND will also allow collection of more data on the efficacy and safety of FMT and likely further support its use. Except for the application of IND, the clinical application of FMT in IBD still faces many issues. Once these issues are addressed, standardized clinical trials can move forward with the hope of not only increasing access to FMT but also developing a well-tolerated and reliable drug that decreases any potential long-term consequences from FMT.

Patients' attitudes toward FMT

Patients are a powerful evolutionary force, whose perceptions and attitudes are important for the clinical application of FMT. A survey showed that up to 95% of adult UC patients and parents of children with UC would consider FMT and are eager for it to become available^[120,121]. Moreover, a long-term follow-up study among patients who had FMT for recurrent CDI showed that 97% of patients were willing to receive another FMT once CDI occurred in the future, and 53% of patients claimed to choose FMT as the first treatment option for substitution of antibiotics^[101]. Another survey reported that when aware of the fecal nature of FMT, 81% of patients still consider it as an alternative treatment for recurrent CDI, especially when recommended by physicians^[122]. Patients recognize the inherently unappealing nature of FMT, but they are nonetheless open to considering it as an alternative treatment for recurrent CDI. Overall, the strong willingness of patients has important implications for the clinical application of FMT and the other microbiota-based treatments in the future.

Synthetic microbiota transplantation

Emerging data showed that two recurrent CDI patients were cured by synthetic microbiota transplantation (SMT) instead of FMT; the synthetic stool was composed of 33 bacterial strains isolated from the feces of an unrelated donor, grown in culture and subsequently administered as a suspension in sterile normal saline^[123]. SMT composed of large numbers of well-defined bacterial strains derived from stools, combined with FMT, can be defined as microbial ecosystem therapeutics^[124]. Recently, the concept of "Robogut" has been proposed^[28], which is actually

a type of chemostat. The chemostat *in vitro* mimics the human gut, even the whole GI tract, and is mainly composed of complex media, a pH and temperature monitor, anaerobic gas, and a stirrer^[28].

For several decades, microbial ecologists have been trying to develop a chemostat resembling the human gut, in which a probiotic gut microenvironment consisting of beneficial anaerobic microbial species by the continuous culture can be manufactured^[125]. SMT based on the chemostat resembling the human gut can be viewed as the next logical step in the development of microbiota therapeutics. For example, if the beneficial bacterial species could be cultured artificially and combined into flora with optimal proportion and magnitude to achieve standardized SMT, the safety and controllability of bacterial origin can be ensured, thus enabling effective quality control and reducing the process of screening donor. Moreover, the synthetic bacteria can be made of freeze-dried powder or capsules in the future to restore the disturbed intestinal microbiota, thereby curing those conditions associated with gut dysbiosis.

CONCLUSION

Evidence from animal and human studies strongly supports the role of intestinal microbiota in the etiology of IBD. The single microbial pathogen in IBD is still undefined, which may not contribute to the onset of IBD. However, many studies based on culture-independent techniques have confirmed aberrant intestinal microbiota and its metabolites in patients with IBD, although whether the intestinal microbiota is the initiating factor in IBD or is secondary to IBD is still not resolved. The present single or combined probiotic products have modest efficacy in IBD treatment; most of which are supplementary therapeutics. FMT is the most radical way to restore the disturbed homeostasis of intestinal microbiota in IBD, but there has been no consensus regarding the selection and screening of donors, the optimal volume and route of administration, pretreatment and preparation before transplantation, efficacy durability and long-term safety profiles. In addition, studies on the mechanisms of FMT for the recovery of intestinal microbial homeostasis and host immunity are still lacking, thus not providing adequate theoretical support for clinical application of FMT.

Currently, FMT application is mainly driven by its remarkable efficacy and the strong demand of clinicians and patients, while institutions have not paid much attention to FMT, especially in technological innovation for the preparation of fecal microbiota products. Moreover, many case series have shown the clinical efficacy of FMT in the management of refractory IBD; several controlled clinical trials have been registered (www.clinicaltrials.gov) and are underway to evaluate the efficacy of FMT in IBD. The hypothesis that whether autologous or allogeneic FMT can induce and maintain persistent remission of mild IBD needs to be confirmed by animal models and pilot clinical trials. At present, data related to the use

and study of FMT to treat IBD other than CDI are still limited. Compared with FMT in CDI treatment, FMT in the management of IBD has to face more rigorous IND application. In addition, SMT as a promising microbiota therapeutic option should also be evaluated rigorously by germ-free and human flora-associated animal models, and the chemostat gut model *in vitro* may also be an excellent technique for the evaluation and production of SMT. Microbiota pathogenesis and therapeutics in IBD is a promising field, and identification and resolution of key issues are imperative to move this field forward.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease

Immunogenetic biomarkers in inflammatory bowel diseases: Role of the *IBD3* region

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Abstract

Many studies have demonstrated the linkage between the *IBD3* region (6p21.1-23), an area which encompasses the famous human leukocyte antigen (HLA) complex, and Crohn's disease (CD) or ulcerative colitis (UC). *IBD3* is the only region that meets genome-wide significance, and provides stronger evidence of the linkage than 16p13.1-16q12.2 (*IBD1*), the locus that contains the susceptibility gene *CARD15*. However, despite these findings, *IBD3* susceptibility genes remain elusive and unclear due to the strong linkage disequilibrium, extensive polymorphism, and high gene density that characterize this area and also due to varying allele frequencies in populations around the world. This area presents an extremely high abundance of genes, including the classical

and non-classical major histocompatibility complex (*MHC*) class I and II genes, and other genes, namely *MHC* class III genes tumor necrosis factor (*TNF*)- α and - β , and *Hsp*, whose proteins play key functions in immunological processes. To date, it is not clear which genes within the *MHC* family contribute to the *IBD* pathogenesis, although certain *HLA* alleles have been associated with *IBD*. Recent insights into the biological function of other genes encoded within the *IBD3* region, such as the *MHC* class I chain-related (*MIC*) genes, have led investigators to a more comprehensive exploration of this region. *MHC* class I chain-related molecule A (*MICA*) is highly polymorphic and interacts with *NKG2D*, its receptor on the surface of NK, $T\gamma\delta$ and $T\ CD8^+$ cells. Increased expression of *MICA* in intestinal epithelial cells and increased expression of *NKG2D* in $CD4^+$ T cells (lamina propria) in patients with CD have also been reported. *MICA* alleles have also been associated with *IBD*, and a variation at amino acid position 129 of the $\alpha 2$ -heavy chain domain seems to categorize *MICA* alleles into strong and weak binders of *NKG2D* receptor, thereby influencing the effector cells' function. In this regard, a relevant role of *MICA*-129-Val/Met single nucleotide polymorphism has recently been implicated in the pathogenesis of *IBD*. *TNF*- α and - β also play an important role in inflammatory response. In fact, *IBD* is commonly treated with *TNF*- α inhibitors. Additionally, polymorphisms of *TNF*- α gene are known to affect the gene expression level and particular *TNF*- α genotypes may influence the response of *IBD* patients treated with *TNF*- α inhibitors.

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Key words: *IBD3*; Tumor necrosis factor; *MICA*; *HLA*; Inflammatory bowel disease

Core tip: This review gathers information about the importance of *IBD3* genomic region in susceptibility to inflammatory bowel disease (*IBD*). The new and old immunogenetic biomarkers of the *IBD3* region (human leukocyte antigen, *MHC* class I chain-related molecule A,

MHC class I chain-related molecule B, and tumor necrosis factor- α and - β) and their role on IBD susceptibility are discussed in the light of recent publications. *IBD3* gene polymorphisms and their clinical relevance for the treatment of the IBD patients are also discussed. Insights into the natural history of these complex diseases may allow in the future appropriate patient selection for early aggressive therapy aimed at modifying the course of the disease.

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INTRODUCTION

Inflammatory bowel diseases (IBD) are an idiopathic disease that seems to involve an immune reaction of the body to its own intestinal tract. The two major types of IBD are ulcerative colitis (UC) and Crohn's disease (CD). Although both UC and CD present different pathologic findings, a significant percentage of patients are classified as indeterminate IBD. Twin studies and segregation analysis strongly support IBD, especially CD, as complex genetic traits^[1], whose etiology also involves immunological and environmental factors. These pathologies are polygenic-related diseases that share some susceptibility loci, but differ at others. A number of studies have been performed to identify IBD susceptibility chromosomal regions, and in some of them causative genes have been found^[2-5]. However, although these studies have indicated multiple regions of interest, the replication of these results has been rather limited. IBD susceptibility genes have classically been identified with different and consecutive names, depending on the chromosomes involved [*e.g.*, 5q31 (*IBD2*), 6p21 (*IBD3*) and 19p (*IBD4*)], while several loci of interest (*e.g.*, at 3p, 3q, 14q and others) require further follow-up.

From a functional point of view, active IBD is immunologically defined as an infiltration of the lamina propria by innate immune cells (neutrophils, macrophages, dendritic and NK cells) and adaptive immune cells (B and T cells). Increased numbers and activation of these cells in the intestinal mucosa enhance local levels of tumor necrosis factor (TNF), located in the *IBD3* region, and other pro-inflammatory interleukins^[3,6]. Cytokines are also essential mediators of the interaction between activated immune cells and non-immune cells, including epithelial and mesenchymal cells. Moreover, germ-line variation in *IL23R*, *IL12B*, *JAK2* and *STAT3* has recently been associated with IBD susceptibility, consistent with the newly described role for IL-23 signaling and Th17 cells in inflammatory disease pathogenesis. Additionally, several

genes involved in different aspects of bacterial handling, such as *NOD2* (from now on called CARD15) on human chromosome 16 (*IBD1*) and the autophagy genes *ATG16L1* and *IRGM*, are defective only in CD. *IL10* and *ECM1* are associated with UC, and an inherited variation at the human leukocyte antigen (HLA) region (*IBD3*) is related to an inflammatory colonic phenotype^[7-16]. In fact, other non-classical *HLA* genes, such as HLA-G and MIC (major histocompatibility complex (MHC) class I related chain), are also implicated in IBD pathology^[4,17,18].

In the last years, the application of genome-wide association studies (GWAS) has been successful in defining the genetic architecture of CD and UC and in delivering genuinely novel and important insights into disease pathogenesis. The recent "ImmunoChip" study^[19], carried out by the International IBD Genetics Consortium, has led to the validation of 163 genetic loci containing susceptibility genes for IBD^[5,20,21], including 71 newly established, a number that is expected to double in the next years. In the "ImmunoChip" study, the maximal genetic association for UC at single nucleotide polymorphism (SNP) rs6927022 mapped adjacent to HLA-DQA1 class II gene (between DQB1 and DRB1). However, there was no evidence of this SNP conferring the risk of developing CD. CD maximal association mapped to HLA class I region and showed no association with UC. These results showed that the HLA region, central to autoimmunity, is especially important and broad-based (influencing a large segment of the at-risk population) to UC. On the other hand, the HLA association with CD seems to be less important and distally located, at least in Caucasians.

Finally, although important obstacles need to be overcome, the successes of the last years suggest that a detailed description of the genetic basis of IBD is a realistic goal. This has unearthed a plethora of attractive targets for the development of future therapeutics. Insights into the natural history of these complex diseases may allow in the future appropriate patient selection for early aggressive therapy aimed at modifying the course of the disease.

In this review, we will analyze the new and old immunogenetic biomarkers of the *IBD3* region and their role on IBD susceptibility, in the light of recent publications. In this context, HLA, MICA, MICB, and TNF- α and - β are the most important genes involved.

HLA SYSTEM AND IBD

Since the first report of an HLA association with IBD in 1972, more than 150 studies investigating the role of *HLA* genes in determining susceptibility and phenotype of IBD have been published^[22]. The HLA complex encodes more than 225 genes, and GWAS have shown consistent evidence of its linkage to *IBD3* (6p21.1-23)^[23-25]. This has been demonstrated in several independent studies of both CD and UC^[26-28]. The structure of this region is shown in Figure 1. The importance of this area was further highlighted by a meta-analysis of ten published genome-wide scans^[3]. *IBD3* was the only locus that met

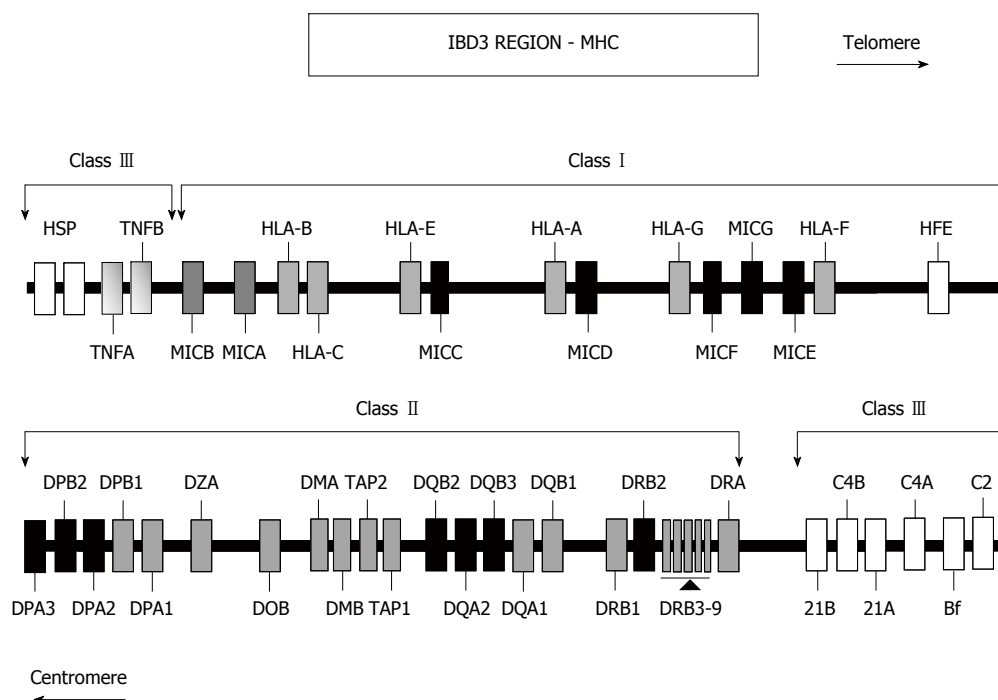


Figure 1 Localization of the *MIC* genes in the major histocompatibility complex class I region on the short arm of human chromosome six. Classical human major histocompatibility complex (MHC) class I locus (HLA-A, -B and -C), non-classical MHC (HLA-E, -F and -G) (grey boxes), and *TNF-α* and HFE (white box) are shown. Pseudogenes are painted in black boxes.

genome-wide significance and provided stronger evidence of linkage than 16p13.1-16q12.2 (*IBD1*), the locus that contains the susceptibility gene *CARD15*. Although it is difficult to estimate the importance of this region in determining the overall genetic susceptibility, calculations derived from studies of HLA allele sharing within families suggest that this region could contribute to 10%-33% of the total genetic risk of CD and 64%-100% of that of UC. Thus, the HLA locus association is far more pronounced for UC than for CD. Before the associations with *NOD2* were discovered, the only consistent genetic associations were those with *HLA* gene polymorphisms, especially of the highly polymorphic HLA class II genes, which are responsible for the presentation of foreign antigens to T-lymphocytes.

In this respect, our group has recently found an increased frequency of *HLA-A*03*, *-DRB1*13*, *-DRB1*01* and *-DRB1*01:03* alleles and a decreased frequency of *DRB1*15* allele in CD patients compared with UC patients^[7]. These data are concordant with previous studies that suggest that, among British patients, *HLA-DRB1*01:03* was associated with the development of severe isolated colonic CD, defined by the requirement for infliximab (anti-*TNF-α*) or colectomy^[1,29], while positive association of CD pathology was found with *HLA-DRB3*03:01* and *-DRB1*13*^[30,31]. The association of *HLA-DRB1*01:03* with UC has also been confirmed in other studies^[32]. Moreover, *HLA-DRB1*15* was also negatively associated with CD^[7], which explains a previous negative association with the serological antigen HLA-DR2 in the 1999 meta-analysis^[31]. This allele seems to confer protection against all subgroups of CD pathol-

ogy, in all ethnic groups, including Japanese. In addition, in our UC patients, allele frequencies of *HLA-DRB1*15* were strongly increased compared with CD patients^[7]. This data agree with other reports showing that *HLA-DRB1*15* is associated with UC in the European^[27], North American^[33], Japanese^[34] and Korean^[35] populations. However, *HLA-DRB1*07* frequency allele, associated in unselected patients with CD in other studies^[1,25,26], was not associated to CD in our study^[7]. Additionally, in contrast to other studies^[31,36-38], we were not able to find an increased frequency in *HLA-DRB1*04* allele in CD patients. However, the frequency of *HLA-DRB1*04* was decreased in UC compared with CD patients^[7]. Additionally, *HLA-DRB1*04* allele was also negatively associated with UC in the Northern European and Japanese populations, in contrast to the positive association with CD^[30].

It was reported that in the Spanish population, the only DR alleles significantly associated with the disease were DR3 (very strong protection) and DR4 (weak protection)^[39]. The strong protective effect of DR3 was evenly distributed among the haplotypes DR3-TNFa1b5, DR3-TNFa2b3, and DR3-TNFother. These results showed the strong protective effect of DR3 in this population, which suggests that the relevant protective gene could be located centromeric to *TNF-α* and *TNF-β* markers.

Recent results showed that IBD Tunisian patients have an increased frequency of the homozygous *DRB1*07* genotype^[40]. In UC patients, *DQB1*03:02* was also predictive of colonic extension, whereas *DRB1*13* and *DQB1*03:01* were associated with limited disease localization (left-sided colitis and proctitis). The same

study showed that *DRB1*15* allele was also increased in patients with extra intestinal manifestations. In CD, female patients showed an increased frequency of the *DRB1*13*, **15*, and *DQB1*06* alleles and *DRB1*13-DQB1*06* haplotype, whereas male patients showed a significant increase of the *DRB1*07*, *DQB1*02* alleles, and *DRB1*07-DQB1*02* haplotype. These recent results suggest a significant association of the homozygous HLA-*DRB1*07* genotype with UC and CD and of several HLA-*DR/DQ* alleles and haplotypes with the clinical phenotypes of these diseases. At the same time, it had also been reported that IBD patients had lower odds of DQ2/8 positivity compared with healthy controls in an Italian population^[41]. Similarly, a pathogenetic link of HLA-B27 between CD and primary sclerosing cholangitis in South Africans has been reported^[42].

HLA-C gene has also been reported to associate with IBD in a study that suggests that HLA-*C*07* and *-C*12* alleles may be strongly associated with the susceptibility to UC and CD, respectively^[43]. Interestingly, GWAS of Japanese population^[44] showed that although HLA-*C*12:02-B*52:01-DRB1*15:02* haplotype increases susceptibility to UC when compared with the frequency of healthy controls, this haplotype reduces the risk of non-colonic CD, whereas it has no effect on colonic CD.

HLA-G is another gene associated with IBD (Figure 1). HLA-G is a non-classical MHC class Ib molecule, predominantly expressed in cytotrophoblasts and, under pathological conditions, in chronically inflamed tissue.

An interesting study investigating a 14-bp deletion polymorphism (Del+/Del-) (rs16375) within exon 8 of the HLA-G gene revealed significant differences between UC and CD^[18]. Heterozygous genotype and the homozygous Del- phenotype were significantly increased, whereas the homozygous Del+ phenotype was significantly decreased when UC was compared with CD. The fact that the deletion in exon 8 of the HLA-G gene seems to influence its transcription activity suggests that the HLA-G gene might play a role in the pathogenesis of IBD.

Other polymorphisms in this region have also been associated with IBD. In this regard, significant association with the rs2395185 variant of the HLA gene and steroid-response, and positive family history in UC patients has recently been reported^[45]. For the HLA-rs2395185 SNP this association persisted both in the adult- and in the pediatric-onset subset of patients with a positive family history.

The unclear role of different HLA molecules in IBDs might be explained by either different affinity to the same peptide or by the HLA molecules binding with different strength to different molecules. In this respect, the IBDs might be explained by molecular mimicry hypothesis^[1,15]. Cross-reactivity of peptides derived from bacterial luminal flora (similar in their structure to HLA antigens) with host “self” antigens present in the gut may lead to the generation of auto-reactive T lymphocytes that either stimulate or inhibit the immune system.

Recent insights into the biological function of other genes encoded within the *IBD3* region have also led investigators to a more comprehensive exploration of this region. An example of this is the analysis of MIC genes presented in the next section.

MIC GENES AND IBD

Polymorphism of MIC Molecules

On the 2 Mb class I segment in the short arm of human chromosome 6, multiple MHC class I chain-related (MIC) loci have been identified^[46,47]. The genes at these loci represent a second lineage of mammalian MHC class I genes. The MIC genes include seven members (MICA to MICG), five of which are pseudogenes and gene fragments, and two, namely MICA and MICB, are functional genes closely related to each other (Figure 1).

They code for stress-induced cell surface molecules, which do not associate with β 2-microglobulin and do not appear to present peptides^[48], since the putative peptide-binding groove is too narrow to accommodate a ligand. This suggests that MICA/MICB are not antigen-presenting molecules^[49]. MICA gene has an overall homology of 83% with MICB gene, but their homology with the classical MHC class I genes is quite low, ranging from 15% to 35%^[50].

MICA/MICB polymorphic residues are positioned on the outer edge of an antigen-binding cleft, apparently bordering an invariant ligand-binding site, unlike MHC class I molecules^[51,52]. The functional significance of these polymorphisms is unknown, although certain changes in the amino acid sequence of the protein influence the abnormal expression^[4,53,54] or the affinity in the interaction with NKG2D/DAP10, its ligand on the surface of NK, $\gamma\delta$ and T CD8+ lymphocytes. MIC triggers the cytotoxicity mediated by these NKG2D-bearing cells (Figure 2). It has been demonstrated that the allelic diversity at the MICA locus affects ligand binding between MICA and NKG2D, potentially affecting NK-cell activation and the modulation of T-cell responses^[4,46,55]. Therefore, the presence of methionine or valine at codon 129 of α 2 domain could confer strong and weak affinity, respectively. Alleles at the MICA locus can be defined as strong or weak binders depending on their capacity to bind NKG2D. The strong NKG2D binding alleles share methionine at position 129, whereas weak binding alleles have valine at this position. In this respect, high-affinity alleles include *MICA*001*, **002*, **007* and **017*, while low-affinity alleles include *MICA*004*, **006*, **008*, **009* and **010*^[51]. Although the significance of high/low affinity for NKG2D in terms of immune activation is unclear, knowledge of specific haplotypes may provide valuable information with regard to modern population's ancestry, admixture and the selective pressures maintaining such as linkage.

The crystal structure of the MICA-NKG2D complex has also revealed that NKG2D binds as a homodimer to one molecule of MICA^[4,55]. One of the NKG2D

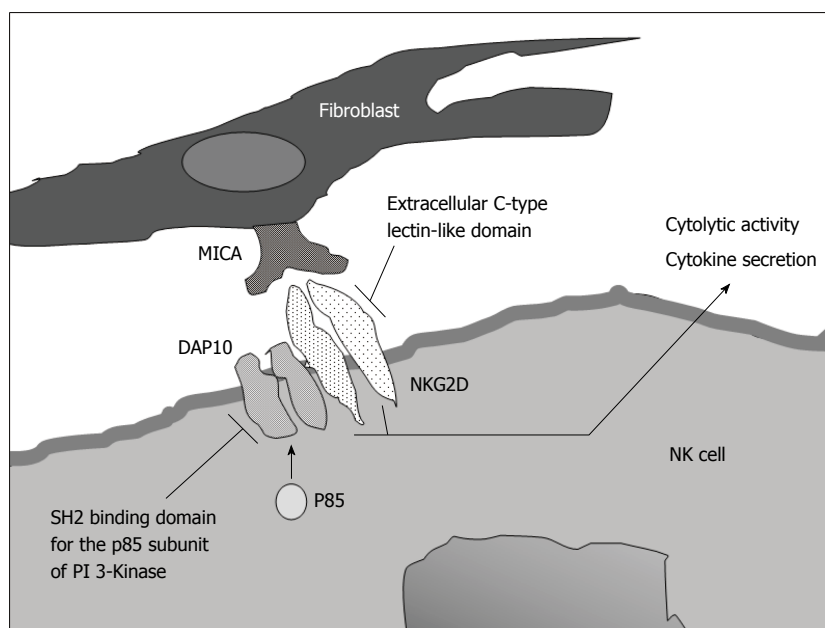


Figure 2 NKG2D signalling requires association with the DAP10 adapter protein. Engagement of NKG2D on NK cells [e.g., via binding of the ligand MHC class I chain-related molecule A (MICA)] can trigger cytolytic activity. It can also elicit cytokine production (e.g., MIP-1 β , TNF- α and IFN- γ)^[7].

molecules binds mostly to the $\alpha 1$ domain of MICA, while the other NKG2D molecule binds mostly to the $\alpha 2$ domain. The NKG2D homodimer overlays MICA diagonally, and $\alpha\beta$ TCR overlays MHC class I molecules in a similar way. The hypothetical binding pockets of MICA remain free of any ligand confirming that MICA is not an antigen-presenting molecule. The half-life for the MICA-NKG2D complex indicates that it is more stable than the complexes formed by TCR and MHC class I molecules^[50].

Furthermore, some authors have also suggested that MICA should be considered a cell homeostasis sensor rather than a cell stress sensor whose up-regulated expression is induced not only by cell distress but also by strong proliferation and pro-inflammatory stimuli that disrupt the cellular homeostasis and elicits a cytotoxicity that eliminates altered cells, thus contributing to the restoration of the normal homeostasis^[56].

MICA has also been shown to play a role in very different aspects of the immune response, such as solid transplant rejection, immune response against viruses and intracellular bacteria, inflammation, homeostasis of epithelia, mother-fetus tolerance and immune response against tumors^[55-59].

MICA/MICB Molecules in Intestinal Pathology

Different studies have demonstrated that MIC genes are implicated in intestinal homeostasis. In fact, MICA is a stress-inducible cell surface antigen that is recognized by intestinal epithelial V $\delta 1$ $\gamma\delta$ T cells, NK cells and CD8⁺ T cells with their NKG2D receptor participating in the immunological reaction in intestinal mucosa. With regard to intestinal pathology, patients suffering active celiac disease with villous atrophy showed a strong MICA expression on the cells of crypts^[60]. MICA is also expressed in villous epithelial cells of the gut in normal or disease-free individuals, but its localization is mostly intracellular.

IL-15, which is over-expressed in the intestine of patients with celiac disease, appears to be involved in this up-regulation of MICA expression and contributes to the cytotoxicity of NKG2D⁺ intraepithelial lymphocytes (IELs)^[61-63]. These cells lyse target epithelial cell lines in a NKG2D-dependent way, developing villous atrophy.

On the other hand, MICA-A5.1 polymorphism has been associated with the risk of atypical celiac disease, while a double dose of MICA 5.1 allele could predispose to celiac disease with gastrointestinal symptoms^[64,65]. Another study has shown that MICB gene is also associated with celiac disease and that both MICB*008 and MICB*002 alleles are part of the celiac disease susceptibility extended haplotypes B8/DR3/DQ2, B18/DR3/DQ2, and DR4/DQ8^[66].

With respect to IBD pathology, increased expression of MICA in intestinal epithelial cells and increased expression of NKG2D in CD4⁺ T cells (lamina propria) in patients with CD have been reported^[67].

Lü *et al.*^[68] investigated the association of the micro-satellite polymorphisms in the intron 1 of MICB and the MICA-MICB haplotype with the susceptibility to UC in the Chinese population. All the subjects were of Chinese Han ethnicity. The frequency of MICB-CA18 was significantly higher in UC patients compared with healthy controls and was increased in the female patients compared with the female healthy controls. Thus, MICB-CA18 seems to be positively associated with UC in the Chinese population.

The same group generated MICA*A5.1-expressing Raji cells by gene transfection in a later study^[69] which also showed that the frequency of MICA*A5.1 was significantly higher in UC patients compared with the healthy controls and that the frequency of a MICA*A5.1/A5.1 homozygous genotype was increased in UC patients. Raji cells with MICA*A5.1 expression produced indeed more soluble MICA than Raji cells with full-length

MICA expression in culture supernatant. Raji cells with MICA*A5.1 expression were more resistant to killing by NK cells than Raji cells with full-length MICA expression. Thus, the *MICA**A5.1 allele and *MICA**A5.1/A5.1 genotype seem to be significantly associated with Chinese UC patients in central China. The authors suggested that *MICA**A5.1 may play a role in the development of UC by producing more soluble MICA and resistance to NK cells. The association of *MICB* exon 2-4 polymorphisms and sMICA expression with the susceptibility to UC in central China was also investigated by the group^[70]. Their results showed that allele frequency of MICB0106 was significantly higher in UC patients than in healthy controls, especially in patients with extensive colitis, moderate and severe disease, extraintestinal manifestations, male patients, and patients over the age of 40 years. The sMICA level was also significantly higher in UC than in healthy controls but not associated with the MICB0106 genotypes. In 2011, they obtained intestinal mucosal biopsies from patients with UC^[71] and observed that the relative amount of MICA mRNA in the colonic mucosa of UC patients was significantly higher compared with controls, as were the *MICB* and *NKG2D* mRNA expressions. Confocal microscopy resonance scanning also showed that MICA was localized predominantly on the basolateral membranes of the epithelium. Further flow cytometry confirmed that the percentage of IFN- γ producer NK cells that expressed *NKG2D* in peripheral blood lymphocytes was higher in UC patients than in the healthy controls. Thus, MICA, *MICB* and *NKG2D* were up-regulated in the colonic mucosa of UC and were associated with activating NK cells with enhanced *NKG2D* and IFN- γ production.

On the other hand, our group found no statistical differences in the distribution of MICA alleles between the IBD, CD and UC patients in the Spanish population, in a recent study^[4]. Our data corroborated the first reported studies showing no association of CD pathology and MICA allele polymorphism and contradict other studies showing that UC is mostly associated with *MICA**007, and Type 2 IBD peripheral arthropathy with *MICA**008^[17,67,72].

The association between the polymorphism of the transmembrane region of MICA and the genetic susceptibility in Tunisian patients with IBD has been recently studied^[73]. No MICA alleles were significantly increased in IBD compared with controls. The *MICA*-A5.1 allele was significantly decreased in CD patients. In UC, *MICA*-A6 was associated with the presence of extraintestinal manifestations, whereas *MICA*-A5 was associated with late age of onset. In CD, *MICA*-A6 was significantly increased in active disease patients when compared with moderately active or inactive disease. In conclusion, MICA seems to play a disease-modifying role, rather than being an important gene in the susceptibility to IBD in the South Tunisian population.

Finally, a recent meta-analysis shows that MICA alleles are associated with ulcerative colitis in Asians^[74].

On the other hand, a variation at amino acid position 129 of the α 2-heavy chain domain (*MICA*-129 Val and Met) seems to categorize *MICA* alleles into strong and weak binders of the *NKG2D* receptor and thereby to influence effector cells' function^[4,53]. Evidence supporting this hypothesis was provided by Zhao *et al.*^[54]. The authors showed that the frequency of *MICA*-129 Val/Val genotype, as well as serum sMICA levels were significantly higher in UC patients than in the controls. Moreover, higher levels of sMICA were associated with severe colitis in the patients.

On the contrary, we have recently found a higher frequency of *MICA*-129 Met/Met and a lower frequency of *MICA*-129 Val/Met genotypes in IBD patients (mainly in UC) compared with control subjects^[4,75]. All these preliminary data suggest a relevant role of *MICA*-129-Val/Met SNP (weak/strong binders of *NKG2D* receptor) in the pathogenesis of IBD^[55]. However, MICA may be in linkage disequilibrium with another gene or genes that are in fact responsible for the protection against IBD, in which case it would involve a secondary disease association. In any case, these facts need to be confirmed by further studies. In conclusion, a relevant role of *MICA*-129-Val/Met SNP (weak/strong binders of *NKG2D* receptor) in the pathogenesis of IBD may imply an association with the protection against the disease, although whether it confers primary or secondary protection remains to be determined.

TNF- α AND TNF- β

TNF- α and - β are key cytokines known to play a role in inflammatory responses, and the loci for these genes are found in the *IBD3* region on chromosome 6p21, which is known to be associated with an increased risk for IBD.

TNF- α /TNF receptor interactions do not only play a pivotal role in the pathogenesis of the inflammatory response, but also cause apoptosis, cell proliferation, and differentiation^[76]. Alterations in the regulation of TNF- α , especially TNF- α over-production, have been implicated in a variety of symptoms associated with autoimmune disorders, including IBD, and especially CD^[77]. Inflammation, anorexia, and weight loss are also all associated with increased levels of circulating TNF- α that are seen in CD^[15].

In fact, three anti-TNF agents, namely infliximab (IFX), adalimumab and certolizumab pegol have been approved by the US Food and Drug Administration for the treatment of luminal CD^[6]. IFX has also been approved for fistulising CD and UC. The advent of these anti-TNF- α agents has also changed the way of treating IBD refractory to standard medications^[76,78]. Clinical trials showed that IFX response varies among patients. It would be beneficial if the efficacy could be predicted by genetic factors since these treatments are very expensive. In this sense, a recent report shows a relationship between the TNF receptor polymorphism and the IFX response in Japanese patients^[78].

Several SNPs in the TNF- α promoter region are known to affect its gene expression (*e.g.*, -238G/A and -308G/A)^[79]. Such variations in the TNF- α promoter region have previously been associated with the susceptibility to a range of autoimmune disorders, asthma, psoriasis and rheumatoid arthritis^[79-82]. The -238G/A SNP is associated with a lower production of TNF- α in patients with ulcerative colitis^[83]. Conversely, the -308A allele is associated with enhanced TNF- α production in cells *in vitro* and in CD patients *in vivo*^[84,85].

The TNF- α -308G/A and the TNF- β NcO1 polymorphisms have also been associated with survival in sepsis or septic shock of various origins. Polymorphisms of the TNF- α -308 and TNF- β +252 do not correlate with age, gender, disease activity or lesion site^[86,87] in the Chinese population.

In a recent study, we did not find differences in individual TNF- α -238G/A and -308G/A SNPs allele and genotype frequencies between CD, UC and IBD patients. These data contrast with other reports showing that the -308 G/A SNP could be associated with IBD susceptibility^[79], while they agree with Cao *et al.*^[86]. A recent meta-analysis showed that TNF- α -308 polymorphisms are also associated with ulcerative colitis in Asians^[74], whereas another study from Northern India [evaluating TNF- α (-1031T > C, -863C > A and -857C > T)] showed that the high-producing genotype of TNF- α (-863AA) was associated with increased risk of IBD, and more so with UC^[88]. Similarly, the combined effect of TNF- α polymorphisms in haplotype analysis demonstrated additionally increased risk of IBD.

However, in the TNF- α promoter gene polymorphism (-308G/A SNP) study, we found an increased frequency of the -308A allele and -308GA genotype in the non-responders to anti-TNF treatment compared with responder patients^[89]. This -308GA genotype has been classified as a high producer of TNF- α cytokine^[80,82], which could explain the different response of IBD patients to TNF- α inhibitor treatment. It can be argued that in situations with a higher production of TNF- α , its inhibitor could not be completely inhibitory, thus promoting a worse clinical response to TNF- α inhibitors. In addition, no differences were found between the two drugs used^[89]. In any case, more polymorphisms affecting the biological response and the responder/non-responder status of anti-TNF should be investigated, since the contribution of these genotypes to the immune response may be weak. It should also be mentioned that no analysis of the biological response of anti-TNF has been performed in this study.

Given the inter-individual variability in the response to the anti-TNF monoclonal antibody IFX, the predictive value of TNF and/or IL1 β as surrogate markers of IFX response was recently studied^[90]. Baseline serum concentrations of TNF and IL1 β were higher in UC patients than in CD patients. CD patients showing < 0.64 pg/mL IL1 β at baseline were more frequently responders than non-responders, and the C allele of the IL1B polymor-

phism was associated with higher IL1 β serum concentrations and with poorer clinical remission after 14 wk of IFX treatment. No significant association was found between serum TNF concentration or TNF polymorphisms and patient response to IFX.

New data on adaptive immunity are emerging, indicating that: (1) the mucosal Th1 and Th2 responses of IBD may be actually secondary to defects of the innate immune response; (2) the dysfunction of regulatory T cells may be contributing to mucosal immune abnormalities; and (3) the newly described Th17 cells are also prominently involved in the gut inflammatory response in both forms of IBD^[15]. In fact, TNF is synthesized by different cells, mostly of monocyte line and T lymphocytes^[6], and also intestinal epithelial cells (IEC). All known responses to TNF are triggered by binding to one of two distinct receptors, designated TNFR1 (also known as TNFRSF1A, CD120a, p55) and TNFR2 (also known as TNFRSF1B, CD120b, p75), which are differentially regulated on various cell types in normal and diseased tissue^[79].

TNF- α induces the expression of various genes, such as urokinase plasminogen activator, cyclooxygenase II (COX II) and vascular endothelial growth factor by activating the nuclear factor- κ B^[91]. In this way TNF- α performs multiple biological functions, such as increasing leukocyte recruitment (induction of leukocyte adhesion molecules)^[92,93], modulation of nitric oxide (NO) production (by increasing vascular permeability)^[94,95], induction of secretion of proinflammatory cytokines^[96], and the proliferation and differentiation of immune cells.

Chronic ulcerative colitis (CUC) is characterized by increased IEC apoptosis associated with elevated TNF, inducible nitric oxide synthase (iNOS), and p53. In addition, p53 has been reported to be increased in crypt IECs in human colitis and is needed for IEC apoptosis in chronic dextran sulfate sodium-colitis^[97]. Goretsky *et al.*^[97] examined the roles of TNF and iNOS in the regulation of p53-induced IEC apoptosis in CUC. The IEC TUNEL staining, caspases 3, 8, and 9, and p53 protein levels, induced by anti-CD3 mAb activation of T cells, were markedly reduced in TNF receptor 1 and 2 gene knockout mice. Induction of IEC apoptosis correlated with increased p53, which was attenuated in iNOS(-/-) mice. IEC p53 levels and apoptosis were also reduced in IL-10 (-/-) colitic mice treated with neutralizing TNF mAb and the iNOS inhibitor, aminoguanidine, further suggesting that TNF and iNOS are upstream of p53 during colitis-induced IEC apoptosis. IEC apoptosis and p53 levels were assessed in control *vs* untreated or anti-TNF-treated CUC patients with equivalent levels of inflammation. In this study, IEC apoptosis and p53 levels were clearly higher in untreated CUC but markedly reduced in patients treated with anti-TNF mAb. Therefore, TNF-induced iNOS activates a p53-dependent pathway of IEC apoptosis in CUC. The inhibition of IEC apoptosis may be an important mechanism for mucosal healing in anti-TNF-treated CUC patients.

However, the mechanisms of action of anti-TNF- α

agents are still unclear^[6,98,99]. The neutralization of TNF- α in the inflamed mucosa is unlikely to be a sufficient explanation. Antibody-dependent cytotoxicity also induces apoptosis or lysis of TNF- α -producing cells^[59,100]. This mechanism involves the Fc portion of antibodies that increases the pro-apoptotic factor caspase-3^[76].

Finally, RNA interference (RNAi) holds great promise for the specific and selective silencing of aberrantly expressed genes, such as TNF- α in IBD. A very recent study investigates the efficacy of an amphiphilic cationic cyclodextrin (CD) vector for effective TNF- α siRNA delivery to macrophage cells and to mice with induced acute-colitis^[101]. RAW264.7 cells were transfected with CD.TNF- α siRNA, stimulated with lipopolysaccharide (LPS). Female C57BL/6 mice were exposed to dextran sodium sulphate (DSS) and treated by intra-rectal administration with either CD. TNF- α siRNA or a control solution. *In vitro*, siRNA in CD nanocomplexes remained intact and stable in both fed and fasted simulated colonic fluids. RAW264.7 cells transfected with CD.TNF- α siRNA and stimulated with LPS displayed a significant reduction in both gene and protein levels of TNF- α and IL-6. CD. TNF- α siRNA-treated mice revealed a mild amelioration in clinical signs of colitis, but significant reductions in total colon weight and colonic mRNA expression of TNF- α and IL-6 compared to DSS-control mice were detected. This data seems to indicate the clinical potential of a local CD-based TNF- α siRNA delivery system for the treatment of IBD.

In sum, a number of IBD immunogenetic markers have been identified^[102-104], and further investigation may establish new approaches to these enigmatic pathologies.

To conclude, further large-scale and functional studies of *IBD3* genes and polymorphisms are required in order to determine specific associated variations within the loci responsible for the gene dysfunction that confers the risk of IBD, and to identify gene variants that may alter the response to the treatment.

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WJG 20th Anniversary Special Issues (17): Intestinal microbiota**Enteric microbiota leads to new therapeutic strategies for ulcerative colitis**

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approach for UC therapy. Various therapies targeting a restoration of the enteric microbiota have shown efficacy in treating patients with active and chronic forms of UC. Such therapies include fecal microbiota transplantation, probiotics, prebiotics, antibiotics, helminth therapy, and dietary polyphenols, all of which can alter the abundance and composition of the enteric microbiota. Although there have been many large, randomized controlled clinical trials assessing these treatments, the effectiveness and safety of these bacteria-driven therapies need further evaluation. This review focuses on the important role that the enteric microbiota plays in maintaining intestinal homeostasis and discusses new therapeutic strategies targeting the enteric microbiota for UC.

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Abstract

Ulcerative colitis (UC) is a leading form of inflammatory bowel disease that involves chronic relapsing or progressive inflammation. As a significant proportion of UC patients treated with conventional therapies do not achieve remission, there is a pressing need for the development of more effective therapies. The human gut contains a large, diverse, and dynamic population of microorganisms, collectively referred to as the enteric microbiota. There is a symbiotic relationship between the human host and the enteric microbiota, which provides nutrition, protection against pathogenic organisms, and promotes immune homeostasis. An imbalance of the normal enteric microbiota composition (termed dysbiosis) underlies the pathogenesis of UC. A reduction of enteric microbiota diversity has been observed in UC patients, mainly affecting the butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, which can repress pro-inflammatory cytokines. Many studies have shown that enteric microbiota plays an important role in anti-inflammatory and immunoregulatory activities, which can benefit UC patients. Therefore, manipulation of the dysbiosis is an attractive

Key words: Ulcerative colitis; Enteric microbiota; Dysbiosis; Probiotic; Fecal microbiota transplantation; Polyphenol

Core tip: The human gut is comprised of a large, diverse, and dynamic microbiota. The enteric microbiota plays an important role in regulating anti-inflammatory and immunoregulatory activities. An imbalance of the normal enteric microbiota composition, termed dysbiosis, underlies the pathogenesis of ulcerative colitis (UC). Therefore, manipulation of the dysbiosis is an attractive strategy for UC therapy. This review discusses new therapies associated with the regulation of enteric microbiota for UC patients.

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INTRODUCTION

Inflammatory bowel disease (IBD) refers to chronic relapsing or progressive inflammatory conditions that may affect the entire gastrointestinal tract. Ulcerative colitis (UC) and Crohn's disease (CD) are two main clinically defined forms of IBD^[1]. In UC, inflammation is limited to the lining of the colon, whereas transmural inflammation occurs along any part of the gastrointestinal tract in CD^[2]. The incidence and prevalence of IBD have continued to increase over the past few decades throughout various regions around the world^[3]. Although the precise pathogenesis is unknown, it is thought that the etiology of IBD involves dysfunction of the mucosal immune system, which develops from complex interactions between the host immune system and genetic and environmental factors^[4].

The gastrointestinal tract is possibly the most complicated immune organ of the entire human body. The intestinal mucosa is continuously exposed to a variety of commensal microbiota and food antigens. The gut must suppress excessive immune responses to antigen stimulation, and thus uses both acquired and innate immune systems to maintain intestinal homeostasis. Evidence suggests that the development and function of the intestinal immune system depend on its specific enteric microbiota, which appears to have co-evolved^[5]. However, a disruption in this system can lead to aberrant immune responses to the enteric microbiota and cause chronic inflammation within the gut^[5,6]. This review focuses on the important role the enteric microbiota plays in maintaining intestinal homeostasis and discusses new therapeutic strategies for UC that are based on the enteric microbiota.

HUMAN ENTERIC MICROBIOTA

The human gut is a complex anaerobic environment with a large, diverse, and dynamic enteric microbiota composed of more than 100 trillion microorganisms - which is ten times greater than the total estimated number of human cells^[7]. Until the 1990s, the characterization of the enteric microbiota was limited to the use of bacteriological culture. However, less than 30% of fecal microorganisms observed under the microscope can be cultured. More recent advances in culture-independent techniques, such as 16S rRNA gene probing-based strategies and metagenomics, have broadened our knowledge of the complexity of this ecosystem^[8,9]. The enteric microbiota is comprised of more than 1000 different bacterial species^[10]. The majority of these organisms are from seven phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Cyanobacteria* and *Actinobacteria*. Over 90% of the human enteric microbiota are from the *Firmicutes* and *Bacteroidetes* phyla, though *Proteobacteria* are also common^[11,12].

There is a beneficial, symbiotic relationship between the host and the enteric microbiota. The host provides a nutrient-rich habitat to support the microbiota, which in

return confers a huge diversity of genes and metabolic functions. First, the enteric microbiota provides nutrition to the host by fermenting non-digestible substrates and producing short chain fatty acids, and aiding in the absorption of ions, amino acids and vitamins. Second, the microbiota provides a barrier against pathogenic bacteria. Furthermore, the microbiota plays an important role in regulating immune and gut homeostasis^[13]. Importantly, an imbalance of this symbiotic state, termed dysbiosis, underlies the pathogenesis of several inflammatory diseases, including allergic skin and respiratory disorders, rheumatoid arthritis, type I diabetes and IBD^[14].

Enteric microbiota in IBD

Quantitative and qualitative changes in the composition of the enteric microbiota have been observed in IBD, through a decrease in the diversity and an increase in the concentration of bacterial species^[13,15]. Dysbiosis in patients with CD has been well characterized, with a loss of bacteria from the *Firmicutes* phylum, including *Faecalibacterium prausnitzii* (*F. prausnitzii*), the major butyrate-producing bacterium in cluster IV of the *Clostridium leptum* phylogenetic group in the gut^[16]. Butyrate, the key energy source for colonic epithelial cells, represses the production of pro-inflammatory cytokines in the intestinal mucosa. The reduction of *F. prausnitzii* in mucosal and fecal samples represents the most replicated species-specific finding so far in CD^[17-19].

The decrease in enteric microbiota diversity has also been described in UC^[20-22]. Researchers found significantly fewer *Bacteroides* and *Clostridium* (*C. coccoides* and *C. leptum*) in fecal samples of UC patients^[23], and a higher amount of *Enterococcus* and *Gammaproteobacteria*^[21,22,24]. Additionally, Machiels *et al.*^[25] found that two well-known butyrate-producing *Firmicutes* bacteria, *Roseburia hominis* and *F. prausnitzii*, were reduced in UC patients. Moreover, the presence of butyrate-producing members of the clostridial cluster was found to vary with disease activity^[26]. Varela *et al.*^[27] found that counts of *F. prausnitzii* were associated with relapse and maintenance of clinical remission, with low counts corresponding to short-term remission, and counts increasing during remission. In another study that analyzed both fecal and biopsy UC specimens, *F. prausnitzii* was sharply decreased in both, whereas *Bifidobacterium* was significantly increased in the biopsy specimens of active UC^[28]. However, the composition of fecal and mucosal-associated microbiota changes throughout the progression of UC^[26,29].

It is still not clear whether the observed dysbiosis contributes to the development or is a consequence of UC. Although studies documenting the association between microbial changes and UC do not necessarily predict cause-effect relationships, they at least demonstrate that the enteric microbiota plays an important role in anti-inflammatory and immunoregulatory activities, which can benefit UC patients^[30].

THERAPIES ASSOCIATED WITH ENTERIC MICROBIOTA

Current therapeutic strategies for treating UC typically follow a step-up strategy, including mesalazine, steroids, immunosuppressants and biologics^[31]. Although a considerable proportion of patients maintain remission when using this approach, a significant number of them experience persistent disease activity and ultimately require colectomy^[32]. Therefore, the development of new and effective therapies is needed. Manipulation of the dysbiosis is an attractive therapeutic approach, particularly because the interaction between enteric microbiota and the host immune system perpetuates the inflammation in UC.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) involves the administration of fecal material from a healthy donor into the intestinal tract of a recipient. This approach attempts to recover the composition and function of the enteric microbiota in patients with chronic gastrointestinal infections and IBD^[33]. FMT was first used in 1985 when standard treatments for *Clostridium difficile* infection (CDI) failed. The efficacy achieved by this application^[34] has since brought greater attention to FMT, which is increasingly being used for treatment of IBD, and UC in particular.

A case report published in 2003 documented six patients with UC who were treated with FMT^[35]. Some symptoms of UC improved within one week, and a complete reversal of symptoms was achieved in all patients by four months after FMT. In addition, there was no clinical, colonoscopic, or histologic evidence of UC in any patient after 1 to 13 years, even without any UC medication. Another study observed ten children with mild to moderate UC for four weeks after receiving FMT^[36]. Seven of these subjects showed a clinical response within one week, and six maintained a clinical response after one month. Among these, three subjects achieved clinical remission after one week, which was maintained throughout the four-week observation period. Furthermore, no serious adverse events were noted.

In contrast, some studies report only temporary clinical improvement from FMT rather than remission. Kump *et al.*^[37] reported short-term clinical improvement with colonoscopic FMT in six patients that were non-responsive to standard medical therapy. Although there were significant changes in the composition of the intestinal microbiota that resulted in a partial improvement of UC-associated dysbiosis, none of the patients achieved clinical remission. Similar results were obtained in a study of five patients with moderate to severe active UC by Angelberger *et al.*^[38]. Only one patient was observed to have some clinical improvement, and none of the five patients achieved remission during the 12-wk study. A transient increase in the similarity to donor microbiota and an increase in phylotype richness were detected in two patients less than four weeks after FMT. Notably, the microbiota

of the sole clinical responder was similar to the donor's, even 12 wk after FMT, which was characterized by successive colonization by the anti-inflammatory and short-chain fatty acid-producing *F. prausnitzii*, *Roseburia faecis* and *Bacteroides ovatus*.

There are several possible explanations for the failure of FMT in some UC patients^[39]. First, enteric microbiota in non-responsive patients may be affected by other factors, including dietary intake or exposure to cigarette smoke. Second, the dysbiosis may be a result of UC, rather than the cause. Finally, the type of patients used in these studies may have affected the results, as those with more severe disease and for whom current medical therapies had failed were chosen.

Because of the uncertain effectiveness and safety issues, the study of FMT in UC is complicated. Further studies should identify the best candidates for FMT, such as patients with mild to moderate UC, those who already have a medically induced remission, or those who are newly diagnosed. In addition, more attention should be paid regarding the side effects accompanying FMT. A recent case report of a patient with UC that had been in remission for more than 20 years experienced a UC flare-up after FMT for treatment of CDI^[40]. Thus, additional careful studies are needed to determine the effectiveness and safety of FMT, and to facilitate the development of FMT as potential therapy for UC patients.

Probiotics and prebiotics

Probiotics are living non-pathogenic microbes, including *Lactobacilli*, *Bifidobacteria*, *Enterococci* and some yeast species^[41], which can benefit the host and affect the structure and function of the enteric microbiota through diverse mechanisms^[42]. Prebiotics, which also confer benefits to the host, are defined as non-digestible, selectively fermented, short-chain carbohydrates that allow specific changes in the composition and/or activity of the enteric microbiota^[43]. Probiotics and prebiotics have been widely used in the prevention and treatment of important gastroenterological conditions, such as irritable bowel syndrome (IBS) and IBD, and infectious diarrhea.

A probiotic mixture named VSL3, consisting of four strains of *Lactobacilli*, three strains of *Bifidobacteria* and one strain of *Streptococcus thermophilus*, was effectively used as maintenance therapy in patients with recurrent or chronic pouchitis^[44-46]. Further evidence supporting the use of probiotics was provided by a one-year, placebo-controlled, double-blind study involving a total of 29 children with active UC who received either VSL3 or a placebo in addition to steroid induction and mesalamine maintenance treatment^[47]. A greater number of patients receiving the placebo relapsed within one year, suggesting that VSL3 is effective in maintaining remission. In two randomized, double dummy trials, Nissle 1917, a non-pathogenic strain of *Escherichia coli* (*E. coli*), was as effective as standard mesalamine in maintaining remission in UC^[48,49]. In both studies, there were no differences in the percentage of patients who relapsed or the safety profile

of one-year treatments with *E. coli* Nissle 1917 or mesalamine. However, an open-label, randomized trial by Zocco *et al*^[50] demonstrated that treatment with *Lactobacillus* GG was more effective than mesalamine in prolonging the relapse-free time in UC patients.

Probiotics also have the capability to induce remission. In a multicenter, randomized, double-blind, placebo-controlled trial including 147 adult patients with mild to moderate UC, a greater percentage of patients receiving VSL3 for 12 wk achieved remission or had greater activity reduction [reduced UC disease activity index (UCDAI) score] than those receiving a placebo^[51]. In a similarly designed clinical trial, 144 patients who received VSL3 for 8 wk in addition to their standard pharmaceutical therapy had greater improvement in UCDAI scores, and a significant reduction in rectal bleeding^[52]. A study by Oliva *et al*^[53] demonstrated that local administration of *Lactobacillus reuteri* ATCC 55730 with standard oral mesalazine reduced the inflammation of rectal mucosa in pediatric UC patients.

The prebiotic germinated barley foodstuff has also been shown to reduce the clinical activity of UC together with the baseline treatment, and to maintain remission^[54-56]. The combined use of probiotics and prebiotics, such as *Bifidobacterium* and galacto-oligosaccharide, has also been shown to improve the clinical status of UC patients^[57]. However, other studies using combinations of probiotics and prebiotics have been less successful^[58,59]. Future probiotic and prebiotic treatments for UC patients should be more personalized, taking into consideration the age of patient, the phase of disease, and the molecular pattern of dysbiosis^[60]. Moreover, therapeutic outcomes may be improved by careful selection of agents, and protective commensal enteric species may be more suitable.

Antibiotics

There have been conflicting results over the past few decades concerning the efficacy of antibacterial therapies for the treatment of UC^[61]. However, a combination of broad-spectrum antibiotics completely inhibited spontaneous colitis in animal models, suggesting a possible rationale for antibiotic combination therapy for the treatment of UC^[61,62]. A two-week antibiotic combination therapy consisting of amoxicillin, tetracycline and metronidazole (ATM) was shown to be effective against *Fusobacterium varium* in UC patients^[63,64]. *F. varium* has been detected in the colonic mucosa of a large number of UC patients^[65] and can induce UC lesions in experimental animals^[66]. ATM treatment was also shown to provide more efficient improvement, remission and steroid withdrawal than a placebo in a double-blind, multicenter trial including 105 patients that was conducted in patients with chronic mild to severe relapsing UC^[67]. This treatment has also shown efficacy in the treatment of refractory or steroid-dependent UC^[68].

Helminth therapy

Modern hygienic practices prevent exposure to helminth

parasites, though epidemiological data suggest that people who carry helminths have fewer immune-mediated diseases^[69,70]. Helminth infections may alter the abundance and composition of enteric microbiota, resulting in a greater proportion of anti-inflammatory strains^[71]. The biocomplexity of the gut, including the interactions between parasites and the enteric microbiota, can influence inflammatory processes^[72]. Studies in rodent models revealed that intestinal parasites and parasite products regulate host immunity and alleviate IBD-like inflammation^[73]. In a randomized, double-blind, placebo-controlled trial involving 54 patients with active UC, a greater number of patients who received 12 wk of treatment with *Trichuris suis* ova showed improvement with no side effects^[74]. Similarly, three patients with active UC in an open trial attained remission with 12-wk *T. suis* ova treatment with no side effects^[75].

Dietary polyphenols

Diet can modulate enteric microbiota composition, with a strong effect on human health^[76]. Polyphenols are members of a large family of plant-derived compounds that have drawn attention due to their antioxidant and anti-inflammatory properties^[77]. Some *in vitro* and *in vivo* studies indicate that dietary polyphenols and their metabolites can interact with the enteric microbiota, and enhance the growth of probiotic bacteria^[78-84]. Due to the prebiotic potential and anti-inflammatory effects, polyphenol supplementation could potentially serve as a complementary medicinal approach to UC. However, there have been no clinical trials evaluating the use of dietary polyphenols for the treatment of UC.

CONCLUSION

As a form of IBD, UC has its own genetic, pathogenic and therapeutic identity. Despite recent advances in UC therapeutic resources, a considerable proportion of UC patients are still refractory to conventional treatment. Dysbiosis is an important immunologic and pathologic process in UC. Accumulating knowledge of dysbiosis has promoted the use of enteric microbiota regulation as a novel promising adjuvant in UC therapy. Therapeutic approaches, such as FMT, probiotics, antibiotics, dietary polyphenols, and helminth therapy, can alter the abundance and composition of enteric microbiota, and improve patient outcomes. However, the safety and effectiveness of bacteria-driven therapies need further evaluation.

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WJG 20th Anniversary Special Issues (3): Inflammatory bowel disease**Risk of infections associated with biological treatment in inflammatory bowel disease**

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Abstract

Tumor necrosis factor- α (TNF- α) inhibitors are biological agents introduced in the late 1990s for the treatment of different immune-mediated diseases as inflammatory bowel disease, rheumatoid arthritis and psoriasis. The most commonly used TNF- α antagonists are infliximab, adalimumab, and certolizumab pegol, and though highly effective in lowering inflammation, the efficacy must be weighed against the potential for adverse events. The treatment-induced immunosuppression is suspected to increase the risk of infections, including the risk of reactivation of latent tuberculosis, as the TNF- α cytokine plays an important role in the immune function. In this topic highlight a short overview of the infection risk associated with TNF- α inhibitor therapy is outlined with a focus on the overall risk of serious infections, mycobacterial infection and latent viral infections.

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Key words: Inflammatory bowel disease; Biological treatment; Tumor necrosis factor- α inhibitors; Risk; Infections; Ulcerative colitis; Crohn's disease

Core tip: The use of tumor necrosis factor- α (TNF- α)

inhibitors are increasing worldwide for the treatment of several chronic immune-mediated diseases as rheumatoid arthritis and inflammatory bowel disease (IBD). As the drugs are relatively new on the market, their long-term safety profile remains uncertain. In particular, a concern regarding an increased infections risk has been debated. In the current topic highlight, a brief overview of the current literature regarding the risk of infections in patients with IBD exposed to TNF- α inhibitors is outlined.

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INTRODUCTION

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD) are relapsing inflammatory conditions affecting the gastrointestinal tract. Since their introduction in the late 1990s, tumor necrosis factor- α (TNF- α) inhibitors have altered the treatment paradigm in IBD and other chronic inflammatory conditions as rheumatoid arthritis and psoriasis. TNF- α inhibitors are chimeric, partly or fully humanized monoclonal antibodies or antibody fragments impairing the pro-inflammatory cytokine, TNF- α . Initially, TNF- α inhibitors were approved for treating fistulizing CD but rapidly their indication expanded and currently, infliximab, adalimumab, certolizumab pegol and golimumab are approved for the treatment of moderate to severe active IBD in adults (certolizumab pegol solely approved for CD and golimumab solely approved for UC)^[1-5], and recently infliximab has been approved for refractory CD and UC in pediatric patients^[6,7]. Assembled, the benefits

of TNF- α inhibitors in the management of IBD are indisputable, but concerns about potential adverse events remain an important issue. TNF- α has a central role in the immune system stimulating the differentiation of monocytes into macrophages, recruitment of neutrophils and creation of granulomas^[8]. Inhibiting this cytokine in the case of inappropriate immune responses as seen in IBD, could potentially impair the effectiveness of the host immune function in the defense against infectious organisms, thereby leading to an increased risk of infections, including the risk of opportunist infections. Shortly after the introduction of infliximab a concern regarding the risk of reactivation of latent tuberculosis (TB) was raised and has led to obligate screening for latent TB prior to TNF- α inhibitor exposure^[9]. Early randomized clinical trials examining the risk of infectious complications have primarily suggested that there is no overall increased risk of infections after treatment with TNF- α inhibitors, but, the true risk profile often relies on ongoing surveillance during the post marketing period with the accumulation of a large number of unselected exposed patients. For this purpose, population- and register-based observational studies are useful, though residual confounding often challenges the interpretation of these studies. Further, drug specific analyses are complicated by the fact that the majority of IBD patients receiving TNF- α inhibitors have concurrent exposure to other immunomodulators as azathioprine and prednisolone, drugs also known to possess an infection risk *per se*^[10-12]. Hence, multiple factors must be taken into account when analyzing and interpretation the infectious risk estimates related to biological treatment.

The aim of the current topic highlight is to give a brief up-date on the existing evidence on the potential increased risk of infections, including the risk of reactivation of latent TB and viral pathogens in IBD patients treated with TNF- α inhibitors.

OVERALL INFECTION RISK

Prior to the approval of infliximab for IBD in the late 1990's, several large, randomized pivotal phase 3 clinical trials were conducted in order to assess the effectiveness of the drug and outline a safety profile. Lichtenstein *et al*^[13] performed a pooled analysis of infections risk based on these sponsor-initiated trials, including the ACCENT I^[3,14], ACCENT II^[15] and SONIC^[1] trials in CD and the ACT I and ACT 2^[2] trials in UC. Authors found that a larger proportion of infliximab- than placebo-treated UC patients had at least one infection (50.1% *vs* 36.3%; $P < 0.001$) whereas the proportion of CD patients treated with infliximab *vs* placebo who had one infection was similar (49.1% *vs* 45.3%; $P = 0.402$). The proportion of IBD patients having one serious infection was similar in infliximab *vs* placebo treated patients (4.7% *vs* 3.7%; $P = 0.427$). Infections expressed as incidences per 100 person-years revealed that infliximab-treated IBD patients had an incidence of 113.80 (95%CI: 109.12-118.62)

vs 115.79 (95%CI: 104.26-128.25) in placebo-treated IBD patients. Similarly, no significant difference between infliximab-treated and placebo-treated IBD patients in incidence of serious infections was observed and authors concluded that infliximab treatment in patients with IBD did not appear to affect incidences of infections. However, the design of randomized clinical trials is often with stringent inclusion and exclusion criteria, and limited follow-up and sample size, hence presenting selected patients populations, that are not optimal for evaluation of the overall, long-term risk of adverse events. In 2009, Fidler *et al*^[16] conducted a single-centre cohort study, evaluating the long-term safety of infliximab in 734 exposed IBD patients followed for a median of 58 mo. The study suggested that the infection rate was similar in IBD patients treated with infliximab compared to IBD patients treated with conventional therapies. In contrast, a prospective, observational study with equivalent follow-up time, based on data from the North American TREAT (Crohn's Therapy, Resource, Evaluation, and Assessment Tool) registry found that infliximab treatment was associated with a significant 43% increased risk of serious infections (HR = 1.43; 95%CI: 1.11-1.84) but authors pointed out that CD severity and use of prednisone and narcotic analgesic carried higher risks, thus the increased risk of serious infection might be attributed to disease severity rather than the infliximab treatment *per se*^[17]. Another North American study including combined data from four large databases in the SABER (Safety Assessment of Biologic Therapy) project investigated the rate of serious infections in patients with different autoimmune disease (IBD, RA, psoriasis, psoriatic arthritis, and ankylosing spondylitis) exposed to TNF- α inhibitor treatment compared with the rate in propensity score matched non-users^[18]. Among 2323 patients with IBD exposed to TNF- α inhibitors no increased risk of serious infections was observed (365 d risk window) following exposure, with an adjusted HR of 1.13 (95%CI: 0.85-1.50) whereas an insignificant trend towards an increased risk was observed for those with concomitant glucocorticoid treatment (> 10 mg/d) with a HR of 1.38 (95%CI: 0.98-1.95). Only for RA patients did the study have sufficient power to analyze the risk of serious infections for the different TNF- α inhibitors separately and stratified analyses revealed that exposure to infliximab was associated with a 25% significant increased risk of serious infections when compared to non-biological treatment (HR = 1.25; 95%CI: 1.07-1.48). There were no increased risk of serious infections related to adalimumab. A small cohort study from Korea compared the risk of serious infections between infliximab and adalimumab in 175 patients with different autoimmune disorders (including 54 with IBD) and found similar infections rates in patients exposed to adalimumab and infliximab but no analyses with a comparison group were performed^[19].

From the Food and Drug Administration Adverse Event Reporting System, Deepak *et al*^[20] studied the association between infections risk and different drugs, in-

cluding TNF- α inhibitors in patients with IBD. Authors found that the risk of serious infections was increased in IBD patients treated with TNF- α inhibitors as monotherapy (OR = 1.95; 95%CI: 1.06-3.59) and further revealed that there was no incremental increase in risk, when combining the treatment with other immunomodulators. A register-based study from the United Kingdom compared the risk of serious infections in patients with rheumatoid arthritis (RA) exposed to TNF- α inhibitors with those exposed to conventional treatments and found a small, significant increased risk related to TNF- α inhibitor exposure (HR = 1.2; 95%CI: 1.1-1.5) with no significant difference between the different TNF- α inhibitors^[21]. Further, when limiting the follow-up to the first 90 days after exposure to TNF- α inhibitors revealed a augmented increased risk of serious infections (HR = 1.8; 95%CI: 1.3-2.6) suggesting that the risk of serious infections is dependent on time since exposure with an increased risk in the early post-treatment period. The study also studied the effect of age on risk of infections and although increasing age was an independent risk factor for serious infections in both treatment groups, there was no difference in relative risk of infection in patients on TNF- α inhibitor therapy in the older population.

To summarize, the overall risk of infections related to TNF- α inhibitor exposure in patients with IBD tend to be limited during long follow-up and mostly driven by patient factors including comorbidities and steroid use. However, the potential for TNF- α inhibitor therapy to improve disease control and allow for steroid dose reduction or elimination, could even out the risk estimates and mask a more substantial increased infections risk related to TNF- α inhibitors *per se*. There is a need of further longitudinal observational studies examining the risk in the different TNF- α inhibitors separately and during different risk windows to assess the potential of a time varying effect.

MYCOBACTERIAL INFECTIONS

According to the World Health Organization, an estimated 8.6 million people developed Tuberculosis (TB) and 1.3 million died from the disease in 2012 making the disease a major global health problem^[22]. Shortly after introduction of infliximab, the concern of reactivation of latent TB in patients receiving TNF- α inhibitors was raised as reports from the Food and Drug Administration Adverse Events Reporting System revealed higher rates of TB in patients exposed to infliximab compared to the background rates^[9]. There were 70 reported cases of TB in patients exposed to infliximab for a median of 12 wk and in 48 patients, TB developed after three or fewer infusions. Of the 70 cases, 91% were from countries with low incidence of TB. The association is further strengthened by the biological plausible link, as TNF- α plays an important role in granuloma formation, which is responsible for the sequestration of mycobacteria. The risk of TB reactivation following TNF- α inhibitor

treatment is mostly studied in patients with rheumatoid arthritis^[23-25] and there is a wide variation in TB rates observed amongst the different studies and a consensus report stated that the relative risk of TB increases 1.6-25 times with exposure to TNF- α inhibitors depending on the clinical context, the drug used and the patient's country of origin^[26]. Interestingly, two studies investigating the risk of reactivation of latent TB in patients with CD did not find any cases of TB and this raises the possibility of a substantial lower risk of TB in patients with IBD compared to those with rheumatoid arthritis, possibly partly because of the younger age of IBD patients^[27,28]. In light of the significant morbidity and mortality associated with TB reactivation, current guidelines demand that patients with IBD who are to be treated with TNF- α inhibitors should first be screened for latent TB with a chest x-ray and tuberculin skin test^[29,30] (in case of a negative test in patients at high TB risk a Quantiferon test should be considered^[29,31-33]). If there is evidence of latent TB infections, patients should begin treatment with isoniazid therapy prior to introducing TNF- α inhibitor treatment.

LATENT VIRAL INFECTIONS

An increased risk of viral infections has also been associated with TNF- α inhibitor exposure as TNF- α has an important role in viral clearance. Particularly the risk of reactivation of varicella zoster virus (VZV), cytomegalovirus (CMV) and viral chronic hepatitis B has received attention.

TNF- α plays a role in the immune response against VZV as it blocks antigen expression and viral virus replication^[34]. Infection with VZV in children causes chickenpox (primary infection) and the infection then becomes latent and may be reactivated in adults causing herpes zoster (shingles). The potential association between TNF- α inhibitors and herpes zoster is mainly studied in patients with RA. A German study including 5040 patients with RA revealed that exposure to the TNF- α inhibitors infliximab and adalimumab was associated with an 82% significant increased risk after adjustment for age, disease severity and glucocorticoid use (HR = 1.82; 95%CI: 1.05-3.15)^[35]. However a more recent study, including 33324 new users of TNF- α inhibitors in patients with RA, IBD, psoriasis, psoriatic arthritis, or ankylosing spondylitis did not find a higher risk of herpes zoster in patients with IBD or other inflammatory diseases who initiated TNF- α inhibitor therapies compared with patients who initiated non-biologic treatment regimens^[36]. As the evidence is not clear, clinicians should be aware of the potential increase risk of herpes zoster, particularly in the light of the high prevalence of VZV seropositive patients. Prior to initiation of TNF- α inhibitor treatment, VZV vaccine may be administered to all adults without evidence of immunity to VZV, but administration of live attenuated VZV vaccine is contraindicated during TNF- α inhibitor therapy and should be given at least 3 wk prior to initiation of any immunosup-

Table 1 Testing to undertake prior to initiation of tumor necrosis factor- α inhibitor treatment

Infection	Tests	Consequences
TB	Latent TB infection should be tested by a combination of patient history, chest X-ray, tuberculin skin test and interferon-gamma release assays (Quantiferon test) according to local prevalence and national recommendations. The Quantiferon test is preferred in patients receiving immunosuppressive therapy and in BCG immunized patients	Patients diagnosed with latent TB infection prior to TNF- α inhibitor exposure should be treated with a complete therapeutic regimen for latent TB. TNF- α inhibitor treatment should not be initiated prior to one month after the introduction of anti-TB treatment When active TB is diagnosed, anti TB-therapy must be started, and TNF- α inhibitor therapy must be stopped but can be resumed after two months if needed
HBV	Blood test for HBsAg, anti- HBsAb and HBcAb to determine HBV status. In patients with positive HBsAg, viremia (HBV-DNA) should also be quantified	Patients with acute HBV infection (HBsAg positive) should be treated with antiviral drugs and treatment with TNF- α inhibitors should not be initiated before a test of HBV DNA is negative In case of evidence of quiescent HBV infection patients should be regularly monitored for evidence of HBV replication during treatment with TNF- α inhibitors
HIV	Blood test for HIV serology	In patients with uncontrolled HIV infection, treatment with TNF- α inhibitors is contra-indicated
VZV	In case of a negative history of chicken pox/herpes zoster infection VZV serology should be tested	Patients with negative serology for VZV should be vaccinated and treatment with TNF- α inhibitor should be awaited three weeks

TB: Tuberculosis mycobacterium; TNF- α : Tumor necrosis factor- α ; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBsAb: Hepatitis surface antibody; HBcAb: Hepatitis B core antibody; HIV: Human immunodeficiency virus; VZV: Varicella zoster virus.

pressant including TNF- α inhibitors.

Cytomegalovirus is another member of the Herpes virus family and has a high seroprevalence in adults^[37]. The primary infection in immune-competent patients is nearly always asymptomatic or comparable to mild mononucleosis-like symptoms but afterwards the infection often becomes latent. Treatment with TNF- α inhibitors has been related to reactivation of latent CMV infections in patients with IBD^[37,38]. The handling of CMV infection in IBD patients remains a challenge as the distinction between active CMV infection and active CMV disease (the presence of both CMV infection and the presence of clinical signs and symptoms, such as fever, leukopenia, or end organ involvement) may be difficult. As there are no current consensus on how to manage active CMV infections/CMV disease in IBD patients treated with TNF- α inhibitors or other immunosuppressant drugs large, controlled trials on antiviral treatment are needed to clearly elucidate the benefits of antivirals in this setting, and to possibly identify risk factors that help identify patients who need antiviral therapy^[39].

The awareness of hepatitis B virus (HBV) infections has increased because of several case reports of severe hepatitis B virus reactivation in patients exposed to TNF- α inhibitors^[40-42]. Although the role of TNF- α in chronic viral hepatitis is limited, there is evidence that TNF- α synergizes with interferons in suppressing viral replication^[43] and is essential in clearing HBV^[44]. Hence, inhibiting the TNF- α cytokine could potentially increase HBV replication leading to active disease. Currently, it is recommended that all patients with IBD are screened for HBV before introducing TNF- α inhibitor therapy and if active HBV replication is detected, antiviral treatment should be administered prior to introducing TNF- α inhibitor therapy.

Table 1 summarizes which tests to undertake prior to initiation of treatment with TNF- α inhibitors.

Based on the current ECCO (European Crohn Colitis Organisation) consensus on preventing opportunistic infections in patients with IBD the following vaccinations are recommended to be given prior to the initiation of TNF- α inhibitors: Influenza (annual), Pneumococcal, Hepatitis B, Varicella, HPV (only women)^[45].

CONCLUSION

There is limited evidence of a substantial increased overall risk of serious infections in patients with IBD exposed to TNF- α inhibitors, however, a small increased risk may not be ruled out, particularly in patients receiving concomitant treatment with corticosteroids and comorbidity. Additionally, it seems that there is an association between exposure to TNF- α inhibitors and different site- and pathogen-specific infections. The risk of reactivation of latent TB infections should be recognized and minimized by TB testing prior to the initiation of TNF- α inhibitor treatment, particularly in countries with high TB incidence. Likewise, it is recommended to screen for latent hepatitis B virus infection and VZV prior to TNF- α inhibitor treatment. The magnitude of the risk of reactivation of CMV in IBD patients exposed to TNF- α inhibitors and other immunosuppressant drugs is not clear and there is no current consensus on how to manage these patients.

In the future, with a growing number of patients exposed to TNF- α inhibitors and hence increasing power, there is need for studies examining the risk of infections following TNF- α inhibitor exposure in patient sub-groups, as for example the elderly. Further there is a need of studies investigating the risk of infections according to specific TNF- α inhibitors separately.

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Ulcerative colitis-associated colorectal cancer

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Abstract

The association between ulcerative colitis (UC) and colorectal cancer (CRC) has been acknowledged. One of the most serious and life threatening consequences of UC is the development of CRC (UC-CRC). UC-CRC patients are younger, more frequently have multiple cancerous lesions, and histologically show mucinous or signet ring cell carcinomas. The risk of CRC begins to increase 8 or 10 years after the diagnosis of UC. Risk factors for CRC with UC patients include young age at diagnosis, longer duration, greater anatomical extent of colonic involvement, the degree of inflammation, family history of CRC, and presence of primary sclerosing cholangitis. CRC on the ground of UC develop from non-dysplastic mucosa to indefinite dysplasia, low-grade dysplasia, high-grade dysplasia and finally to invasive adenocarcinoma. Colonoscopy surveillance programs are recommended to reduce the risk of CRC and mortality in UC. Genetic alterations might play a role in the development of UC-CRC. 5-aminosalicylates might represent a favorable therapeutic option for chemoprevention of CRC.

cer; Risk factor; Dysplasia; Surveillance colonoscopy; Chemoprevention

Core tip: Colorectal cancer (CRC) is more frequent in patients with long-term ulcerative colitis (UC), and is one of the most serious and life threatening consequences of UC. Knowledge of risk factors for CRC is important to identify UC patients who need surveillance. Colonoscopy surveillance programs are recommended to reduce the risk of CRC and mortality in UC. Genetic alterations might play a role in the development of CRC in UC patients. 5-aminosalicylates might represent a favorable therapeutic option for chemoprevention of CRC.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease showing mucosal inflammation from the rectum to the oral side. Crohn and Rosenberg reported the first case of adenocarcinoma complicating UC in 1925^[1]. The risk of developing colorectal cancer (CRC) is found to be high in patients with long-term UC^[2,3]. UC-CRC is considered to develop from a non-neoplastic inflammatory epithelium to dysplasia to cancer. Therefore, colonoscopic surveillance in patients with long-standing UC has been recommended. UC-CRC shows characteristic clinicopathological features of CRC. In this paper, the characteristic properties of UC-CRC are reviewed.

CLINICAL FEATURES OF COLITIS-ASSOCIATED CRC

Clinicopathological features

UC-CRC patients are younger, more frequently have mul-

multiple cancerous lesions, and a macroscopically permeating pattern of spread, including mucinous or signet ring cell carcinomas, compared with sporadic CRC^[1-3]. The advanced stage at presentation causes less favorable outcome of UC-CRC in IBD patients.

Incidence of UC-CRC

An inflammatory environment is believed to play an important role in the pathogenesis of UC-CRC in patients with chronic colitis^[4]. UC-CRC accounts for about 1% of all CRC^[5]. The risk of CRC begins to increase 8 or 10 years after the initial diagnosis^[6-8]. Table 1 summarizes representative reports on the risk of developing colorectal cancer in patients with UC. Eaden *et al*^[9] conducted a meta-analysis of 116 studies, and found that the probability of CRC in patients with UC increased with the duration of disease; 1.6% at 10 years after a onset of UC, 8.3% at 20 years after, and 18.4% at 30 years after. This increased incidence of UC-CRC is four to ten times greater than that for sporadic CRC, and the average age of onset is 20 years earlier. Several other studies reported that the risk of UC-CRC in UC patients was 5%-7% at 20 years after onset of disease^[10-14], 7%-14% at 25 years^[15,16] and 7.5%-18%^[9,17] at 30 years. Eaden *et al*^[9] confirmed that there is an increased risk for UC-CRC in pancolitis (5.4%), while the incidence in all patients with UC was 3.7%. In some countries, patients with UC have not been found to be at increased risk of CRC development. Winther *et al*^[18] reported that the probability of CRC in Denmark was 0.4% by 10 years, 1.1% by 20 years, and 2.1% by 30 years of disease, suggesting that neither the overall cancer risk nor the UC-CRC risks increased after a median of 19 years of follow-up evaluation. This low rate of CRC development may reflect the high rates of surgical approach and chemoprevention for UC in Denmark. Taken together, the 5-aminosalicylic acid (5-ASA) treatment and frequent surveillance colonoscopy with proctocolectomy for dysplasia could explain the reduction in the incidence of CRC in UC patients. Moreover, current studies indicate that the risk of CRC seems to be lower. Rutter and co-workers reported cumulative incidences of UC-CRC of 2.5% at 20 years of colitis duration, 7.6% at 30 years, and 10.8% at 40 years^[19] indicating only a 1.5 to 2-fold increased risk for CRC (5%) in comparison with the non-UC population. Söderlund *et al*^[20] indicated that the overall cumulative incidence of CRC at 10, 20, and 30 years after the inflammatory bowel disease (IBD) diagnosis was 1%, 1.5%, and 2.7%, respectively. Manninen *et al*^[21] reported only slightly increased risk for UC-CRC in UC patients in a Finnish cohort. Hata *et al*^[22] reported that the cumulative risks for the development of invasive cancer at 10, 20, and 30 years were 0.5%, 4.1%, and 6.1%, respectively, while those for the development of definite dysplasia at 10, 20, and 30 years were 3.1%, 10.0%, and 15.6%, respectively. A further current systematic review with meta-analysis in 2014, based on 81 studies and 181923 patients, reported that the risk of UC patients

developing colorectal cancer has decreased steadily, and the incidence rate decreased from 4.29/1000 patient-years in the 1950s to 1.21/1000 patient-years in the last decade^[23]. The risk of developing CRC with longstanding Crohn's colitis is considered to be similar to that of UC, while the incidence of CRC in Crohn's disease showed various ranges in cancer risk^[24-27].

Patients with UC who have undergone proctocolectomy have a very small risk of dysplasia in the ileal pouch^[28]. Anal transitional zone dysplasia after ileal pouch-anal anastomosis is infrequent. Anal transitional zone preservation did not lead to the development of cancer in the anal transitional zone after five to ten years of follow-up^[29].

Patients with UC who develop CRC have a worse prognosis than for CRC patients without UC^[30-33], and the long-term prognosis of UC-CRC is even worse when patients with the same tumor stage are compared^[32]. UC-CRC is frequently diagnosed at an advanced stage^[33]. These findings emphasize the importance of knowledge of risk factor for UC-CRC and surveillance for patients with UC.

RISK FACTORS OF UC-CRC

Knowledge of risk factor for CRC is important to categorize subgroups of UC patients who need frequent surveillance or intense treatment. Risk factors for CRC in UC patients include, anatomical extent, young age at diagnosis, duration of disease, concurrent primary sclerosing cholangitis (PSC) and family history of CRC. In addition, smoking, pseudopolyps, persistent inflammation of the colon and backwash ileitis are also risk factor for CRC^[34,35]. These UC patients with risk factors should be enrolled in an intensive surveillance program.

Pancolitis

The anatomical extent of colitis is an independent risk factor for the development of CRC. A meta-analysis showed that the incidence of CRC in patients with extensive UC was 5.4%^[36]. Patients with pancolitis are at high risk of CRC, left-sided colitis is moderate risk, and proctitis and proctosigmoiditis are low risk, being similar to the non-UC population^[20,37,38]. Ekbom *et al*^[38] reported that UC patients with pancolitis had a 15-fold higher risk of CRC compared with the non-UC group, in contrast to an increased risk of 2.8 for patients with left-sided colitis and no significant increased risk for those with proctitis, and reported an overall risk of 4.8 for UC patients with extensive disease.

Young age

Young age at onset of colitis has been reported as an independent risk factor for CRC^[6]. CRC risk varied by age at initial diagnosis of UC; patients diagnosed at childhood (0-19 years old) had a relative risk of 43.8 followed by those diagnosed in young (20-39 years old) with a relative risk of 2.65^[39].

Table 1 Risk of developing colorectal cancer in patients with ulcerative colitis

Ref.	Year	Years after UC						Country
		10	15	20	25	30	40	
Gilat <i>et al</i> ^[14]	1988	0.2%	2.8%	5.5%		13.5%		Israel
Lennard-Jones <i>et al</i> ^[13]	1990		3%	5%				United Kingdom
Langholz <i>et al</i> ^[7]	1992				3.1%			Denmark
Eaden <i>et al</i> ^[9]	2001	1.6%		8.3%		18.4%		United Kingdom
Hata <i>et al</i> ^[22]	2003	0.5%		4.1%		6.1%		Japan
Winther <i>et al</i> ^[18]	2004	0.4%		1.1%		2.1%		Denmark
Lakatos <i>et al</i> ^[17]	2006	0.6%		5.4%		7.5%		Hungary
Rutter <i>et al</i> ^[107]	2006			2.5%		7.6%	10.8%	United Kingdom
Söderlund <i>et al</i> ^[20]	2009	1%		1.5%		2.7%		Sweden

UC: Ulcerative colitis.

Long disease duration

Duration of UC is an important risk factor for CRC development. Among patients with IBD, the median time from diagnosis of IBD to CRC was 17 years; 21% of IBD patients developed tumors within 10 years after onset^[40].

PSC

IBD patients with PSC, a chronic cholestatic liver disease, have an increased risk of CRC^[41]. Broomé *et al*^[42] revealed a cumulative risk of CRC in UC patients with PSC of 9% after 10 years duration of symptoms, 31% after 20 years and as high as 50% after 25 years; compared with 2%, 5% and 10% in patients with UC alone matched for each duration. A meta-analysis found that 21% of UC patients with PSC developed CRC compared with 4% of UC patients without PSC. The risk of CRC in UC patients with PSC was 4.8-fold higher than that in patients with UC without PSC^[43].

Family history of colorectal cancer

A family history of CRC in UC patients increases the risk of CRC, irrespectively of the type and extent of IBD, as compared with patients with UC without positive family history for CRC^[44-46].

MOLECULAR FEATURES OF UC-CRC

Genetic characteristics detected in sporadic CRC, such as genetic mutations, microsatellite instability (MSI), and DNA hypermethylation, were also recognized in UC-CRC^[4,33,47-50]. Mutations in *p53* occur early in the adenoma-carcinoma sequence and are often detected in non-dysplastic or indefinite dysplasia in UC, while *p53* mutations occur in the late phase in sporadic adenoma^[51]. MSI is also relatively frequent in non-dysplastic inflamed epithelia, and transforming growth factor β receptor type II (*TGF β RII*) is one of genes targeted by the MSI process in UC-CRC^[50]. Hyper-methylation of *hMLH1*^[50,52], *p16INK4a*^[53], and *p14ARF*^[54] seems to precede dysplasia and contribute to the genetic alterations in UC-CRC^[55]. MicroRNAs (miRNAs) play a critical role in regulating key pathogenic mechanism in IBD^[56]. The miRNA-124a

gene has a tumor-suppressive function and is methylated during carcinogenesis in UC patients, and the methylation level of miR-124a-3 is a promising marker for estimating individual risk for CAC^[57]. By contrast, miRNA-155 overexpression is particularly associated with MSI in CA-CRC^[58]. These molecules might be useful biomarkers for early detection and treatment response of CRC in IBD patients.

Inflammatory stresses, such as reactive oxygen species and some free radicals, may cause these genetic changes^[59-61] and are considered to be factors in the pathogenesis of UC-CRC^[62,63].

SURVEILLANCE COLONOSCOPY

Cancer surveillance is based on the high-risk factors that identify patients who are likely to develop cancer. The management of UC has changed with biological therapies, surgical treatment, and surveillance tools, which have reduced the risk of CRC in patients with UC^[9,20,23]. Surveillance is recommended during the remission state to reduce the difficulty of differentiating reactive change from dysplasia^[64]. Data from an 18-year surveillance program demonstrated that cancer was detected at an early stage in 80% of surveyed patients, compared with only 41% of non-surveyed UC patients^[65]. There is evidence that surveillance colonoscopy reduces the risk of CRC and mortality in UC: The overall 5-year survival rate was 77% for the surveillance group, compared with only 36% for the control group^[33,35,65]. It has been reported that a prior history of surveillance colonoscopy reduces the odds of developing CRC by 60%-80%^[35,66].

These guidelines, commissioned by the Clinical Services' Committee of the British Society of Gastroenterology for clinicians and allied professionals caring for patients with IBD in the United Kingdom, provide an good clinical practice for surveillance and treatment^[67]. The guidelines state that UC patients should be advised to have a review colonoscopy 8-10 years after disease onset to check the extent of colitis. Current recommendations are for regular surveillance every 1-2 years in the second decade of the disease to yearly by the fourth decade. The recommended guidelines for the surveillance of CRC in

Table 2 Timing of surveillance colonoscopy for colorectal cancer in ulcerative colitis

Ref.	Year	Guidelines	Beginning of surveillance (years after onset of symptoms)	Surveillance schedule
Van Assche <i>et al</i> ^[73]	2013	European Crohn's and Colitis Organization (ECCO)	8 yr	High risk ¹ ; 1-2 yr Low risk ¹ ; 3-4 yr
Farraye <i>et al</i> ^[72]	2010	American Gastroenterological Association (AGA)	8 yr	Extensive colitis or left-sided colitis; 1-2 yr Patients with PSC; 1 yr High-grade or low-grade dysplasia; colectomy or repeat colonoscopy within 6 mo Indefinite dysplasia; 3 to 12 mo No dysplasia; 1-2 yr
Kornbluth <i>et al</i> ^[70]	2010	American College of Gastroenterology (ACG)	8-10 yr	1-2 yr
Cairns <i>et al</i> ^[68]	2010	British Society of Gastroenterology (BSG)	10 yr	lower risk ² ; 5 yr intermediate risk ³ ; 3 yr higher risk ⁴ ; 1 yr
Leighton <i>et al</i> ^[69]	2006	American Society for Gastrointestinal Endoscopy (ASGE)	8-10 yr	1-2 yr (indefinite dysplasia: 3 to 6 mo)
Eaden <i>et al</i> ^[71]	2002	United Kingdom	8-10 years (pancolitis) 15-20 yr (left-sided colitis)	3 yr (second decade) 2 yr (third decade) 1 yr (fourth decade)

¹Low-risk is 0-2 points and high-risk is 3-4 points; Risk factor: Pancolitis, endoscopic and/or histological inflammation, pseudopolyps, and family history of CRC; each risk factor is counted with one point; ²lower risk: extensive colitis with no active inflammation or left-sided colitis; ³intermediate risk: extensive colitis with mild active inflammation or post-inflammatory polyps or family history CRC in FDR aged ≥ 50 ; ⁴higher risk: active inflammation or stricture in past 5 years or dysplasia in past 5 years declining surgery or PSC/transplant for PSC or family history CRC in FDR aged < 50 . PSC: Primary sclerosing cholangitis; CRC: Colorectal cancer; FDR: First-degree relatives.

UC by some societies^[67-75] are summarized in the Table 2. These recommend surveillance programs are summarized as follows: (1) surveillance colonoscopy should be performed during remission state; (2) initial surveillance colonoscopy for CRC should be performed 8-10 years after onset; (3) regular surveillance should be performed annually or biannually; (4) surveillance colonoscopy for patients with PSC should be performed annually from the beginning of PSC diagnosis; (5) random biopsy of four lesions might be taken every 10 cm through the colon; and (6) if dysplasia is detected, the biopsies should be reviewed by a second gastrointestinal pathologist.

The main aim of surveillance programs is to detect dysplastic alterations. The cumulative probability of developing dysplasia or CRC in UC patients was 7.7% at 20 years and 15.8% at 30 years^[13]. CRC incidence was 14 of 1000 UC patients-years' duration and the incidence of any advanced lesion was 30 of 1000 person-years' duration. When low-grade dysplasia (LGD) is detected on surveillance, there is a 9-fold risk of developing cancer and 12-fold risk of developing any advanced lesion^[76]. Among patients with LGD who undergo colectomy, 19% will already harbor CRC or high-grade dysplasia (HGD) and 30%-50% will develop advanced neoplasia over the following 5 years^[77-79]. HGD carries a 43% risk of synchronous cancer^[80].

The guidelines described random biopsy^[67]. A study

of multiple biopsies taken at colonoscopy suggested that 33 biopsies are required to give a 95% chance of detecting dysplasia^[81]. In contrast, targeted biopsies are recommended to increase the frequency of dysplasia detection, compared with random biopsies. Chromoendoscopy might improve the imagining of subtle mucosal changes that are suggestive of neoplasia, compared with standard endoscopy^[82]. Indigo carmine contrast dye highlights irregularities in the mucosal architecture, improving the precision of endoscopic diagnosis. Methylene blue stains the normal epithelium of the colon; the absence of staining might indicate the presence of neoplastic changes in the intestine. Magnifying endoscopy could assist us to further visualize the delicate surface patterns^[83].

On the other hand, some studies have highlighted the failures of surveillance colonoscopy by the guidelines^[39,84,85]. In 50%-80% of cases with colitis-associated neoplasms, the lesions are not visible upon endoscopy^[38]. It would be necessary to clarify that the surveillance systems could contribute to the decline of the mortality of UC patients.

TREATMENT FOR DYSPLASIA

Histopathological diagnosis of polypoid mucosa of UC is important with respect to clinical treatment for dysplasia. UC with HGD usually leads to a total colectomy because

of the high incidence of adenocarcinoma (42%-67% of the colectomy specimens)^[75,79,86]. When HGD in flat mucosa is the initial discovery, surgery or polypectomy is done. Polypectomy should be performed along with biopsies taken from the surrounding mucosa. If the polypectomy is confirmed as complete and biopsies of the adjacent mucosa are negative for dysplasia, a follow-up examination within 6 mo should be performed^[75,78]. If the dysplastic lesion persists or “dysplasia associated lesions or masses” (DALM) exists, a proctocolectomy should be performed^[75].

In contrast, the management of LGD is controversial^[87]. About 30%-50% of patients with LGD progressed to HGD or CRC; an unrecognized synchronous CRC may already be present in up to 20% of UC patients with LGD^[77,79], which indicates that LGD is a risk factor for CRC. In contrast, some studies have shown that patients with LGD have a lower rate of CRC than previously reported^[88].

Dysplasia found in DALM or in areas without any macroscopically visible mucosal alteration is believed to be the origin CRC^[89,90]. The guidelines also state that particular attention should be paid to DALM that harbor a high risk of progression to CRC^[71]. In addition, patients with DALM are recommended to undergo prophylactic proctocolectomy with an ileoanal pouch. By contrast, some polyps, such as adenoma-like mass (ALM), are unrelated to colitis and can be managed by endoscopic polypectomy because of less carcinogenic potential^[91].

The serrated neoplasia pathway was recently proposed in CRC^[92]. Serrated epithelial changes and sessile serrated polyps are uncommonly detected (0.2%-1%) by colonoscopy in chronic ulcerative colitis and Crohn's disease patients^[93], while Bossard *et al*^[94] found that serrated lesions, such as hyperplastic polyps and sessile serrated polyps/adenomas, accounted for approximately 7% of premalignant lesions in the inflamed mucosa of patients with IBD.

CHEMOPREVENTION

Chemoprevention refers to the use of an anti-inflammatory therapy or other substance to reduce or prevent the development of cancer. The current decreased incidence of CRC might be due to a better control of inflammation by improved medical therapy and higher rates of mucosal healing^[95]. Intervening before the development of neoplasia might be promising method to decrease cancer and prevent colectomy.

5-ASA

5-ASA, the nuclear kappa-B pathway inhibitor, is a first line agent for anti-inflammatory therapy^[96]. Continuing inflammation is a plausible mechanism causing malignant transformation; therefore, anti-inflammatory therapy might be useful for chemoprevention in UC patients. 5-ASA reduces oxidative stress, inhibits cell proliferation and promotes apoptosis^[96]. Most reports indicated that

5-ASA reduces the risk of CRC in chronic ulcerative colitis^[34,35,97,98], however, a few did not. A meta-analysis performed by Herrinton *et al*^[85] showed a protective association between the use of 5-ASA and CRC or a combined end point of CRC/dysplasia: in a pooled analysis of 334 CRC cases among patients with UC, regular use of 5-ASA reduced the risk of CRC by approximately 50%, similar to the regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) in patients without UC^[99]. In contrast, several studies did not find any chemopreventive effect of 5-ASA^[100-102].

Ursodeoxycholic acid

Ursodeoxycholic acid (UDCA) may be a practical chemoprevention against colonic exposure to bile acid in patients with PSC^[103]. UDCA use was closely associated with decreased prevalence of neoplasia because UDCA reduces the colonic concentration of the secondary bile acid as a carcinogen^[104,105]. It has been reported that UDCA reduced the risk of CRC in PSC patients with IBD by 80%^[103].

Steroids, aspirin, NSAIDs

There are several studies that suggest steroids, aspirin, and NSAIDs may reduce the incidence and mortality of CRC in UC^[34,35,106].

Total colectomy

The cumulative CRC risk in patients with UC is 30%-40% at 20-30 years after onset of disease, which might suggest that total colectomy is recommend after 15 years of disease in patients with UC. However, the role for prophylactic colectomy in patients with IBD remains controversial.

FUTURE DIRECTION

Accumulating studies about UC-CRC suggest that control of long-term background inflammation and mucosal damage is vital. The use of maintenance chronic ulcerative colitis therapies could be an important strategy for reducing CRC risk in UC patients. Inflammatory stresses, such as reactive oxygen species and some free radicals, have been considered to cause genetic damages of UC epithelium. UC-CRC shows characteristic clinicopathological features. Analysis of the correlation between these genetic features and clinicopathological features might be useful to develop new therapies and to reduce the risk of UC-CRC in the future.

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Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs

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Abstract

The intestinal microbiota is the collection of the living microorganisms (bacteria, fungi, protozoa, and viruses) inhabiting the gastrointestinal tract. Novel bacterial identification approaches have revealed that the gastrointestinal microbiota of dogs and cats is, similarly to humans, a highly complex ecosystem. Studies in dogs and cats have demonstrated that acute and chronic gastrointestinal diseases, including inflammatory bowel disease (IBD), are associated with alterations in the small intestinal and fecal microbial communities. Of interest is that these alterations are generally similar to the dysbiosis observed in humans with IBD or animal models of intestinal inflammation, suggesting that microbial responses to inflammatory conditions of the gut are conserved across mammalian host types. Studies have also revealed possible underlying susceptibilities in the innate immune system of dogs and cats with IBD, which further demonstrate the intricate relationship between gut microbiota and host health. Commonly identified microbiome changes in IBD are decreases in bacterial groups within the phyla *Firmicutes* and *Bacte-*

roidetes, and increases within *Proteobacteria*. Furthermore, a reduction in the diversity of *Clostridium* clusters XIVa and IV (*i.e.*, *Lachnospiraceae* and *Clostridium coccooides* subgroups) are associated with IBD, suggesting that these bacterial groups may play an important role in maintenance of gastrointestinal health. Future studies are warranted to evaluate the functional changes associated with intestinal dysbiosis in dogs and cats.

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Key words: Microbiome; 16S rRNA; Inflammatory bowel disease; Probiotic; Dog; Cat

Core tip: Several studies in dogs and cats have demonstrated that acute and chronic gastrointestinal diseases, including inflammatory bowel disease (IBD), are associated with alterations in the small intestinal and fecal microbial communities. Of interest is that these alterations are generally similar to the dysbiosis observed in humans with IBD or animal models of intestinal inflammation, suggesting that microbial responses in inflammatory conditions of the gut are conserved across mammalian host types, and dogs and cats may serve as models to study therapeutic approaches to spontaneous inflammatory conditions of the gastrointestinal tract.

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INTRODUCTION

The intestinal microbiota is the collection of the living

microorganisms (bacteria, fungi, protozoa, and viruses) inhabiting the gastrointestinal (GI) tract. Novel bacterial identification approaches have revealed that the gastrointestinal microbiota of dogs and cats is, similarly to humans, a highly complex ecosystem, comprising at least several hundred different bacterial phylotypes^[1-3]. It has been suggested that the intestine of mammals is home to a total of 10^{10} - 10^{14} microbial cells, which is approximately 10 times more than the number of host cells. This complex microbial ecosystem and its interplay with eukaryotic host cells have a significant impact on health and disease of dogs and cats. The stimulation of the host immune system and the microbial metabolites produced by the resident microbiome are thought to be one of the most important driving forces behind the coevolution of gastrointestinal microbiota with their host. Gut microbes aid the host by acting as a defending barrier against enteropathogens. They also aid in digestion of complex fiber sources and produce various short-chain fatty acids and other metabolites that provide nutritional support for enterocytes, and which play an important role in the development and regulation of the host immune system^[4,5].

Several studies in dogs and cats have demonstrated that acute and chronic gastrointestinal diseases, including inflammatory bowel disease (IBD), are associated with alterations in small intestinal and fecal microbial communities^[6-14]. Of interest is that these alterations are generally similar to the dysbiosis observed in humans with IBD or animal models of intestinal inflammation^[15-20], suggesting that microbial responses in inflammatory conditions of the gut are conserved across mammalian host types, and dogs and cats may serve as models to study therapeutic approaches to spontaneous inflammatory conditions of the gastrointestinal tract. Recent data support this model, as it has been shown that for example probiotic products (*i.e.*, VSL#3 strains) show similar clinical benefits in dogs with IBD as have been previously demonstrated in humans^[21].

Studies have also revealed possible underlying susceptibilities in the innate immune system of dogs and cats with IBD, which further demonstrates the intricate relationship between gut microbiota and host health^[10,22-25]. Currently, a major hurdle for a more detailed understanding of host-microbe interactions in dogs and cats is the fact that to date most studies evaluating microbiota in GI diseases have examined only a single time point or have evaluated only a small number of diseased animals.

Yet the possibility to alter the microbiome holds promise as a therapeutic mean in veterinary medicine, and recent studies would confirm that direct or indirect manipulations of the intestinal microbiome *via* antibiotics, diet and/or probiotics may have beneficial effects in gastrointestinal diseases of dogs and cats^[21,26-29].

INTESTINAL MICROBIOTA IN HEALTHY DOGS AND CATS

Various studies have evaluated the bacterial communities

in healthy dogs and cats using either traditional bacterial culture or novel next-generation sequencing approaches. Based on traditional bacterial culture, the small intestine of dogs and cats harbors generally low bacterial counts, ranging between 10^2 to 10^5 cfu/g of small intestinal content; however, some studies have identified much higher counts in healthy dogs and cats with up to 10^9 cfu/g^[30,31]. Cats appear to have higher counts of anaerobic bacteria compared to dogs in the proximal small intestine^[31]. The total bacterial count in the colon ranges between approximately 10^9 and 10^{11} cfu/g and the most abundant cultivable groups are *Bacteroides*, *Clostridium*, *Lactobacillus*, *Bifidobacterium*, and *Enterobacteriaceae*^[32,33]. Next-generation sequencing studies of the 16S rRNA gene have described the canine and feline microbiome, which on higher phylogenetic levels resembles the microbiome of humans and other mammals. On average, 10 different bacterial phyla have been identified in the feline and canine gut, with *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Actinobacteria* making up the vast majority of all gut microbes^[1,3,11,34-36]. Minor abundant members are the phyla *Tenericutes*, *Verrucomicrobia*, *Cyanobacteria*, and *Chloroflexi*. The *Firmicutes* contain various sequences affiliated with *Clostridium* cluster IV and *Clostridium* cluster XIVa and these are together with *Bacteroides* or *Prevotella* the predominant bacterial groups in fecal samples^[3,35,37]. *Helicobacter* are the predominant group in the stomach (> 90% of sequencing reads)^[38], while the duodenum is home to *Enterobacteriaceae*, *Clostridiales*, *Bacteroidales*, and *Lactobacillales*^[36].

The canine^[39] and feline^[40,41] fecal metagenomes (*i.e.*, shotgun sequencing of genomic DNA) have also been studied. This approach yields information regarding microbial genes present in a sample, and allows assessment of the functional capabilities of the microbiota, summarized in Figure 1. Despite variation in the microbial populations of cats and dogs, the functional capabilities are noted to be highly conserved.

More detailed overviews about the canine and feline microbiota in healthy animals have been reported previously^[42-45].

MICROBIOME IN GASTROINTESTINAL DISEASES OF DOGS AND CATS

In recent years, the GI microbiota has garnered strong interest due to the potential etiopathologic role in host health and disease. Many studies in humans and animal models have suggested that various GI disorders are associated with alterations of the GI microbiota. While specific enteropathogens have been recognized in cats and dogs (*i.e.*, *Campylobacter jejuni*, *Clostridium difficile*, *Clostridium perfringens*, and *Salmonella*), most of them are found in similar frequency across healthy animals. Therefore, their cause-effect relations remain unclear^[46,47]. It is now well recognized that more broad changes in the intestinal microbiome are associated with acute and chronic GI disease. Examples of recent studies in companion ani-

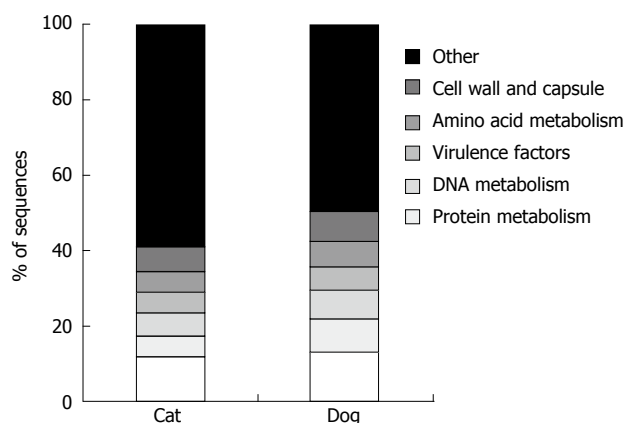


Figure 1 Relative proportion of major microbial gene functions in cats and dogs. Metagenomic data adapted from Swanson *et al.*^[39], Barry *et al.*^[40], and Tun *et al.*^[41].

imals and their findings are summarized in Table 1. The cause-effect relationships of the alterations are still being elucidated, but especially in chronic enteropathies such as IBD there is now strong evidence that the gut microbiota plays an important part in the pathogenesis of the disease. Studies in humans have shown an association between IBD and microbial dysbiosis in the intestine. In these studies, a decrease in the bacterial phyla *Firmicutes* and *Bacteroidetes*, and an increase in *Proteobacteria* and *Actinobacteria* were associated with IBD^[16]. Furthermore, a reduction in the diversity of *Clostridium* clusters XIVa and IV (*i.e.*, *Lachnospiraceae* and *C. coccoides* subgroups) are associated with IBD, suggesting that this bacterial group may play an important role in maintenance of gastrointestinal health, possibly due to production of short chain fatty acids (SCFA). Similar studies have now been reported in dogs and cats with IBD, and a comparison of the observed microbial shifts for humans, dogs, and cats with IBD is provided in Table 2.

Microbiota alterations in canine and feline IBD

In veterinary medicine, chronic enteropathies with intestinal inflammation are commonly seen in dogs and cats. The response to treatment is used to allow for distinction of different types of enteropathies, such as food-responsive diarrhea, antibiotic-responsive diarrhea, and steroid-responsive diarrhea. Idiopathic IBD is a subgroup of enteropathies and it is defined as an inflammation of the GI tract with persistent or recurrent GI signs due to unknown cause^[48]. To diagnose IBD, known causes for GI inflammation need to be excluded. Therefore, empirical treatments are applied sequentially, starting with a dietary trial, followed by antibiotic therapy if there is a lack of response to diet, and finally, treatment with anti-inflammatory drugs, if response to previous treatments was inadequate. Similarly to human IBD, the exact pathogenesis of canine/feline IBD is unknown, but is suspected to be the result of an abnormal interplay between an altered intestinal microbiota, an underlying genetic susceptibility of the host, and dietary and/or en-

vironmental factors^[48]. Consequently, several studies have revealed possible underlying susceptibilities in the innate immune system of dogs and cats with chronic GI inflammation. These include altered differential expression of Toll-like receptors (TLR)-2 and 4^[25,49], single nucleotide polymorphisms that lead to hyper-responsiveness of TLR-5 to flagellin in German Shepherd dogs (GSDs)^[22], and decreased expression of CD11c(+) cells in dogs with IBD^[50]. There is also well known anecdotal evidence that certain breeds are more prone to chronic GI inflammation. In addition to GSDs, which have been shown to possess polymorphisms in the TLR-4 and TLR-5 genes that are significantly associated with IBD^[51], other dog breeds such as Rottweiler, Border Collie, Boxer dog, and Weimaraner have been shown to possess increased risks for developing IBD^[23]. Of those breeds, breed specific studies evaluating the association between mucosa-adherent microbiota and intestinal inflammation were performed only in GSDs and Boxer dogs. In GSDs with chronic intestinal inflammation, the mucosa-adherent microbiota were analyzed in small intestinal brush samples and showed a significant over-representation of *Bacilli* and *Erysipelotrichi* when compared to healthy Greyhound dogs^[6]. Interestingly, this is somewhat different to the results observed in other studies where more diverse populations of dogs with chronic intestinal inflammation were evaluated. In these studies, the most frequently observed changes in the mucosa-adherent microbiota in the small intestine were increases in members of the *Proteobacteria*, especially *Escherichia coli*-like organisms^[9] or *Pseudomonas*^[8], with concurrent decreases of members of *Firmicutes* and *Bacteroidetes*. In a more recent study evaluating mucosa-adherent microbiota in the duodenum of dogs with IBD by next-generation sequencing, the proportions of *Fusobacteria*, *Bacteroidaceae*, *Prevotellaceae*, and *Clostridiales* were significantly increased in healthy dogs. In contrast, specific bacterial genera within *Proteobacteria*, including *Diaphorobacter* and *Acinetobacter*, were either more abundant or more frequently identified in dogs with IBD^[7]. One study evaluated specifically the presence of *Mycobacterium avium* subspecies *paratuberculosis* in duodenal biopsies of dogs with IBD or intestinal neoplasia by qPCR and reported that 19% of diseased dogs were PCR positive for this organism^[52]. Less published information is available about the mucosa-adherent microbiota of cats with IBD. While sequencing methods have not yet been reported for the characterization of feline IBD, a study using fluorescent *in situ* hybridization (FISH) has revealed an increase in *Enterobacteriaceae* in duodenal biopsies of cats with IBD^[10]. Furthermore, a relationship between increased bacterial numbers and the severity of histological inflammation was observed^[10].

Several studies have evaluated the fecal microbiota in dogs and cats with chronic GI disease. In one study, cats with IBD had lower FISH counts for total bacteria, *Bacteroides* spp., and *Bifidobacterium* spp., but higher counts of *Desulfovibrio* spp. compared to healthy cats^[53]. *Desulfovibrio* spp. are a sulfate-reducing bacterial group and able to

Table 1 Reported microbial shifts in dogs and cats with gastrointestinal disease

Ref.	Species	Sampling location	Animal (sample size)	Method	Microbial changes in diseased animals
Suchodolski <i>et al</i> ^[1] , 2012	Dog	Duodenal biopsies	IBD (<i>n</i> = 14) HC (<i>n</i> = 6)	454-pyrosequencing (16S rRNA gene)	Increase in <i>Proteobacteria</i> (<i>Diaphorobacter</i> , <i>Acinetobacter</i>) Reduction in <i>Fusobacteria</i> , <i>Bacteroidaceae</i> , <i>Prevotellaceae</i> , <i>Clostridiales</i>
Suchodolski <i>et al</i> ^[1] , 2010	Dog	Duodenal biopsies	IBD (<i>n</i> = 7) HC (<i>n</i> = 7)	Gene clone libraries (16S rRNA gene)	Increase in <i>Proteobacteria</i> Decrease in <i>Clostridia</i>
Allenspach <i>et al</i> ^[6] , 2010	Dog	Duodenal brushings	Chronic enteropathies (<i>n</i> = 13) HC (<i>n</i> = 8)	Gene clone libraries (16S rRNA gene)	Increase in <i>Actinobacteria</i> , <i>Lactobacillales</i> , <i>Erysipelotrichales</i>
Xenoulis <i>et al</i> ^[9] , 2008	Dog	Duodenal brushings	IBD (<i>n</i> = 10) HC (<i>n</i> = 9)	Gene clone libraries (16S rRNA gene)	Increase in <i>Enterobacteriaceae</i> (<i>E. coli</i>); Reduction in biodiversity
Suchodolski <i>et al</i> ^[36] , 2008	Dog	Duodenal brushings	Chronic enteropathies (<i>n</i> = 71) HC (<i>n</i> = 64)	Gene clone libraries (fungal ITS gene)	No significant differences in fungal communities
Glanemann <i>et al</i> ^[52] , 2008	Dog	Stomach, duodenum, Colon biopsies	Chronic GI disease (<i>n</i> = 42) HC (<i>n</i> = 14)	PCR	Presence of <i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i> Detected in 8/42 (19%) of dogs with chronic GI disease
Manchester <i>et al</i> ^[59] , 2013	Dog	Colon biopsies	Granulomatous colitis (<i>n</i> = 6)	FISH	Presence of invasive <i>E. coli</i>
Simpson <i>et al</i> ^[58] , 2006	Dog	Colon biopsies	Granulomatous colitis (<i>n</i> = 13) HC (<i>n</i> = 38)	FISH	Intracellular translocation of adherent and invasive <i>E. coli</i>
Rossi <i>et al</i> ^[21] , 2014	Dog	Fecal samples	IBD (<i>n</i> = 20) HC (<i>n</i> = 10)	qPCR (16S rRNA gene)	Decreased in <i>Faecalibacterium</i> spp. And <i>Turicibacter</i> spp.
Foster <i>et al</i> ^[62] , 2013	Dog	Fecal samples	Acute diarrhea (<i>n</i> = 7) HC (<i>n</i> = 12)	454-pyrosequencing (18S rRNA gene)	No significant differences in fungal communities
Suchodolski <i>et al</i> ^[14] , 2012	Dog	Fecal samples	IBD (<i>n</i> = 19) AHD (<i>n</i> = 13) NHD (<i>n</i> = 12) HC (<i>n</i> = 32)	454-pyrosequencing (16S rRNA gene) qPCR (16S rRNA gene)	AHD: most profound alterations in their microbiome Increase in <i>Sutterella</i> , <i>Clostridium perfringens</i> Decrease in <i>Blautia</i> , <i>Ruminococcaceae</i> , <i>Turicibacter</i> IBD: Decrease in <i>Faecalibacterium</i> spp., <i>Fusobacteria</i>
Markel <i>et al</i> ^[55] , 2012	Dog	Fecal samples	Chronic enteropathies (<i>n</i> = 87) AHD (<i>n</i> = 48) HC (<i>n</i> = 180)	qPCR (16S rRNA gene)	Decrease in <i>Faecalibacterium</i> spp., <i>Turicibacter</i> spp., <i>Ruminococcaceae</i> Increase in <i>C. perfringens</i> and <i>E. coli</i>
Jia <i>et al</i> ^[65] , 2010	Dog	Fecal samples	Chronic diarrhea (<i>n</i> = 9) HC (<i>n</i> = 8)	FISH	Increase in <i>Bacteroides</i>
Glanemann <i>et al</i> ^[52] , 2008	Dog	Fecal samples	Diarrhea (<i>n</i> = 4) HC (<i>n</i> = 9)	T-RFLP	Increase in <i>C. perfringens</i> , <i>E. faecalis</i> , and <i>E. faecium</i>
Ghosh <i>et al</i> ^[13] , 2013	Cat	Ileum full-thick biopsies	Severe systemic ill (<i>n</i> = 50) HC (<i>n</i> = 50)	FISH PCR	Increase in <i>E. faecalis</i> Attachment of <i>E. coli</i> to intestinal epithelial cell
Janeczko <i>et al</i> ^[10] , 2008	Cat	Small intestine biopsies	IBD (<i>n</i> = 17) HC (<i>n</i> = 10)	FISH	Increase in <i>Enterobacteriaceae</i>
Abecia <i>et al</i> ^[54] , 2010	Cat	Fecal samples	IBD (<i>n</i> = 8) HC (<i>n</i> = 10)	FISH	No significant differences in specific bacterial population
Inness <i>et al</i> ^[53] , 2007	Cat	Fecal samples	IBD (<i>n</i> = 11) HC (<i>n</i> = 34)	FISH	Decreased total bacteria, <i>Bifidobacterium</i> spp. and <i>Bacteroides</i> Increase in <i>Desulfovibrio</i>

IBD: Inflammatory bowel disease; HC: Healthy control; AHD: Acute hemorrhagic diarrhea; NHD: Non-hemorrhagic diarrhea; FISH: Fluorescence *in situ* hybridization; T-RFLP: Terminal restriction fragment polymorphism; qPCR: Quantitative polymerase chain reaction.

produce hydrogen sulfides, which may be associated with the pathogenesis of feline IBD. However, another study did not identify significant differences in FISH counts

between cats with IBD and controls, although the same bacterial groups were targeted^[54]. A recent study utilized 454-pyrosequencing of 16S rRNA genes to describe

Table 2 Comparison of reported microbial shifts in inflammatory bowel disease relative to healthy subjects across species

Organism	Human (Crohn's disease)	Human (ulcerative colitis)	Dog	Cat
Firmicutes	Decreased ^[15,18,56] 1,2,4,5	Decreased ^[15,18] 1,2,4	Decreased ^[7] 1,6	
Class Clostridia			Decreased ^[7,8,14] 1,2,4,6,7	
Family Ruminococcaceae (Clostridial cluster IV)	Decreased ^[15] 2,4	Decreased ^[15] 2,4	Decreased ^[7,8,14] 1,2,4,6,7	
Family Lachnospiraceae (Clostridial cluster XIVa)	Decreased ^[15,18] 1,2,4,5	Decreased ^[15,18] 1,2,4,5	Decreased ^[7,8] 1,6,7	
Bacteroidetes	Decreased ^[18] 1,4	Decreased ^[18] 1,4	Decreased ^[7,9] 1,6,7; increased ^[8] 1,7	
Genus Bacteroides			Decreased ^[7] 1,6	Decreased ^[53] 1,5
Fusobacteria			Decreased ^[7,8] 1,6,7	
Proteobacteria	Increased ^[18] 1,4	Increased ^[18] 1,4	Increased ^[7-9] 1,6,7	Increased ^[10] 1,5
Family Enterobacteriaceae	Unchanged ^[18] 1,4; increased ^[20] 2,5	Unchanged ^[18] 1,4; decreased ^[20] 2,5	Increased ^[7-9] 1,6,7	Increased ^[10] 1,5
<i>E. coli</i>	Unchanged ^[15] 2,4; Increased ^[19] 1,4,5		Increased ^[8] 1,7	
Adherent-Invasive <i>E. coli</i>	Increased ^[19] 1,5		Increased ^[58] 1,3,5	
Actinobacteria	Increased ^[18] 1,4	Increased ^[18] 1,4	Increased ^[7] 1,6	
<i>Mycobacterium avium</i> subspecies <i>pseudotuberculosis</i>	Controversial ^[18] 1		Increased ^[52] 1,4	
Genus Bifidobacterium	Decreased ^[15] 2,4	Decreased ^[15] 2,4		Decreased ^[53] 1,5

¹Based on mucosal samples; ²Based on fecal samples; ³Granulomatous colitis in Boxer dogs and French bulldogs; ⁴Quantitative polymerase chain reaction;

⁵Fluorescent *in situ* hybridization; ⁶454-Pyrosequencing; ⁷Gene clone libraries. *E. coli*: *Escherichia coli*.

changes in fecal microbiota in cats with chronic diarrhea and their response to dietary modifications^[29]. Several bacterial groups correlated with improved fecal scores after therapeutic response to diet. Those included *Slackia* spp., *Campylobacter upsaliensis*, *Enterobacteriaceae* Raoultella spp., *Collinsella* spp., and unidentified genera within *Clostridiales* and *Lachnospiraceae*^[29].

More data about the fecal microbiota are available in dogs. In one study, fecal samples from healthy dogs, dogs with acute non-hemorrhagic diarrhea, dogs with acute hemorrhagic diarrhea, and dogs with active or therapeutically controlled idiopathic IBD were analyzed by sequencing of the 16S rRNA gene^[14]. Dogs with acute diarrhea, especially those with acute hemorrhagic diarrhea, had the most profound changes in bacterial groups in their microbiome. Dogs with acute hemorrhagic diarrhea had significant decreases in *Blautia*, *Ruminococcaceae* including *Faecalibacterium*, and *Turicibacter* spp., and significant increases in genus *Sutterella* and *C. perfringens* compared to healthy dogs. In another recent study, the fecal microbiome of healthy dogs, dogs with chronic enteropathies, and dogs with acute hemorrhagic diarrhea was evaluated by qPCR assays for selected bacterial groups^[55]. The most pronounced changes were decreases in *Faecalibacterium* spp., *Turicibacter* spp., and *Ruminococcaceae* in CE and AHD. *E. coli* and *C. perfringens* were significantly increased in CE and AHD^[55]. Especially *Faecalibacterium* spp. is an important group that frequently appears depleted in canine GI disease. This has been confirmed in another study evaluating the fecal microbiota of dogs with idiopathic IBD, in which *Faecalibacterium* spp. was the major bacterial group decreased in diseased dogs^[21]. Noteworthy, *Faecalibacterium* spp. correlated with improvement in clinical activity index, suggesting that *Faecalibacterium* spp. may be important for canine GI health, and also may be useful as a monitoring marker for improvement of fecal dysbiosis^[14,21].

While the above discussed studies have reported

changes in microbial groups in GI disease of dogs and cats, only limited information is available about the metabolic consequences that are associated with this dysbiosis, as currently no comprehensive functional studies have been reported in dogs or cats. Alterations in the composition of intestinal microbiota are thought to be an important factor in the pathogenesis of chronic GI diseases. It can be hypothesized that the observed microbiome changes may lead to altered intestinal barrier function, damage to the intestinal brush border and enterocytes, an increased competition for nutrients and vitamins, and to an increased deconjugation of bile acids. Of interest is that commonly depleted groups in GI disease are *Lachnospiraceae*, *Ruminococcaceae*, and *Faecalibacterium*. These bacterial groups, important producers of SCFA, may play an important role in maintenance of gastrointestinal health, as their depletion leads to decreased production of SCFA (e.g., butyrate, acetate), which may impair the capability of the host to down-regulate aberrant intestinal immune response. The importance of some of these bacterial groups that are depleted in IBD have recently been demonstrated in humans. For example, *Faecalibacterium prausnitzii* is consistently reduced in human IBD and this bacterium has been shown to secrete metabolites with anti-inflammatory properties, thereby down-regulating interleukin (IL)-12 and interferon gamma and increasing IL-10 secretions^[56]. Disturbances may result in a dysregulation of adaptive immune responses, and lead to inflammation and/or reduced activity against infection. Also, some bacteria produce various toxic agents such as ammonia, D-lactate, endotoxin (LPS), or exotoxin (enterotoxin), and compete for vitamins or other nutrients. Consequently, depletions in serum vitamin B12 concentrations and also increases in serum concentrations of D-lactate are potential consequences of intestinal dysbiosis in cats^[57]. However, more comprehensive metabolomics studies are needed in companion animals to elucidate the consequences of

the dysbiosis observed in GI disease.

Invasive and adherent bacteria

A specific form of colitis occurs in Boxer dogs^[58] and occasionally also in French Bulldogs^[59]. This disease is termed granulomatous colitis. Microbiota analysis based on sequencing of 16S rRNA genes in combination with FISH has revealed invasive bacteria in the colonic mucosa of Boxer dogs with granulomatous colitis. Based on comparative 16S rRNA gene analysis, these bacteria have high phylogenetic similarity to *Escherichia coli* (*E. coli*) and *Shigella*. *In situ* analysis with 16S rRNA gene based FISH probes against *E. coli* showed multifocal clusters of invasive bacteria within macrophages in the colonic mucosa^[58]. The eradication of these invasive *E. coli* in Boxer dogs and French Bulldogs with granulomatous colitis correlates with clinical remission, inferring a causal relationship between these bacteria and the disease^[59]. Of interest is that these observed phylotypes of *E. coli* isolated from Boxer dogs have high phylogenetic resemblance to *E. coli* associated with Crohn's disease in humans^[16,59]. The breed specific predisposition of Boxer dogs and French bulldogs to *E. coli* associated granulomatous colitis highly suggests the presence of a genetic susceptibility that impairs their ability to fend off adherent and invasive *E. coli*.

Bacteria invading the intestinal mucosa may also be part of neutrophilic IBD in other dog breeds. Due to the recognized association of granulomatous and neutrophilic IBD with invasive bacteria, specialized testing based on FISH has been developed that allows localizing the bacteria in intestinal biopsies for better guidance of treatment decisions^[59].

ALTERATIONS IN FUNGAL MICROBIOTA

While bacteria are by far the most abundant constituents of the mammalian GI tract, it is now recognized that the gut harbors a highly diverse population of fungal organisms. FISH and shotgun sequencing studies of human and canine fecal DNA have estimated the abundance of fungal organisms and archaea as < 2% of total microbiota^[39,60]. A recent metagenomic approach estimated that the feline GI microbiota constitutes 0.02% fungi, 0.09% archaea, and 0.09% viruses^[41]. Fungi were described using pyrosequencing of the fungal 18S rRNA gene in pooled fecal samples of cats^[3], with *Aspergillus* and *Saccharomyces* being the most abundant fungal genera. A study reported the prevalence and identification of fungal organisms in the small intestine of healthy dogs and dogs with chronic enteropathies^[61]. The results indicated a high prevalence (up to 76.1% of dogs) and high diversity of fungal organisms in the canine duodenum. Furthermore, dogs with gastrointestinal disease harbored opportunistic fungal pathogens. A total of 51 different phylotypes were identified, with the most frequently observed phylotypes being *Pichia* spp., *Cryptococcus* spp., *Candida* spp., and *Trichosporon* spp.^[61].

A recent study has characterized the fungal micro-

biome (mycobiome) of 19 dogs (12 healthy dogs and 7 dogs with acute diarrhea) using fungal tag-encoded FLX-Titanium amplicon pyrosequencing^[62]. Five distinct fungal phyla were identified, with *Ascomycota* (median: 97.9% of obtained sequences) and *Basidiomycota* (median 1.0%) being the most abundant. A total of 219 fungal genera were identified across all 19 dogs with a median (range) of 28 (4-69) genera per sample. *Candida* was the most abundant genus found in dogs. However, no significant differences were observed in the relative proportions of fungal communities between healthy and diseased dogs. Therefore, additional studies are needed to elucidate the importance of fungi on intestinal health and disease of animals.

CONCLUSION

Studies using molecular approaches have provided clear evidence for alterations in microbial communities in the small and large intestine of dogs and cats with GI disorders. However, currently there is a lack of comprehensive studies evaluating the functional consequences of these alterations. A better understanding of these mechanisms will allow for the development of treatment modalities (*e.g.*, prebiotics, probiotics, metabolites) aiming at modulating microbial communities and their produced metabolites. Anecdotal case reports have reported some success using fecal transplantation in dogs with chronic diarrhea. Results of initial studies suggest that the administration of probiotic strains can be useful in dogs with GI disease. For example, probiotic strains have shown benefits in dogs with IBD^[21], puppies with acute parvoviral enteritis^[63], and adult dogs with non-specific diarrhea^[26,27]. In cats, probiotics strains have been shown to be beneficial in cats with chronic diarrhea^[28] and stress-related diarrhea in a shelter environment^[64]. However, future studies will need to evaluate how these microbial changes impact the immune and metabolic status of dogs and cats.

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Dismicrobism in inflammatory bowel disease and colorectal cancer: Changes in response of colocytes

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(CRC) that is a common disease of high economic costs in developed countries. The CRC has been increasing in recent years and its mortality rates are very high. Multiple biological and biochemical factors are responsible for the onset and progression of this pathology. Moreover, it appears absolutely necessary to investigate the environmental factors favoring the onset of CRC and the promotion of colonic health. The gut microflora, or microbiota, has an extensive diversity both quantitatively and qualitatively. *In utero*, the intestine of the mammalian fetus is sterile. At birth, the intestinal microbiota is acquired by ingesting maternal anal or vaginal organisms, ultimately developing into a stable community, with marked variations in microbial composition between individuals. The development of IBD is often associated with qualitative and quantitative disorders of the intestinal microbial flora (dysbiosis). The healthy human gut harbours about 10 different bacterial species distributed in colony forming units which colonize the gastrointestinal tract. The intestinal microbiota plays a fundamental role in health and in the progression of diseases such as IBD and CRC. In healthy subjects, the main control of intestinal bacterial colonization occurs through gastric acidity but other factors such as endoluminal temperature, competition between different bacterial strains, peristalsis and drugs can influence the intestinal microenvironment. The microbiota exerts diverse physiological functions to include: growth inhibition of pathogenic microorganisms, synthesis of compounds useful for the trophism of colonic mucosa, regulation of intestinal lymphoid tissue and synthesis of amino acids. Furthermore, mucus seems to play an important role in protecting the intestinal mucosa and maintaining its integrity. Changes in the microbiota composition are mainly influenced by diet and age, as well as genetic factors. Increasing evidence indicates that dysbiosis favors the production of genotoxins and metabolites associated with carcinogenesis and induces dysregulation of the immune response which

Abstract

Patients with inflammatory bowel disease (IBD) have an increased risk of 10%-15% developing colorectal cancer

promotes and sustains inflammation in IBD leading to carcinogenesis. A disequilibrium in gut microflora composition leads to the specific activation of gut associated lymphoid tissue. The associated chronic inflammatory process associated increases the risk of developing CRC. Ulcerative colitis and Crohn's disease are the two major IBDs characterized by an early onset and extraintestinal manifestations, such as rheumatoid arthritis. The pathogenesis of both diseases is complex and not yet fully known. However, it is widely accepted that an inappropriate immune response to microbial flora can play a pivotal role in IBD pathogenesis.

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Key words: Dismicrobism; Inflammatory bowel disease; Colorectal Cancer; Dysbiosis; Eubiosis; Heat shock proteins

Core tip: Dysbiosis could be the common denominator of inflammatory bowel disease (IBD) and colorectal cancer. A well balanced gut microbiota promotes the health of colocytes through the production of important compounds and the correct modulation of immune system. Qualitative and quantitative modifications in the bacterial composition are responsible of changes in the biochemistry and in the cell cycle of colocytes. The aim of this work is to focus on the molecular mechanisms that connect dysbiosis, IBD and colorectal cancer. Experimental studies are oriented towards the discovery of new probiotic-based therapies for the treatment and prevention of inflammatory and carcinogenetic processes.

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INTRODUCTION

Inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and Ulcerative colitis (UC), are pathologies characterized by a chronic inflammation of the gastrointestinal tract. In UC, rectal and descending colon involvement is a typical feature whereas CD can affect any part of the gastrointestinal tract from the mouth to the anus. Their aetiopathogenesis is not yet fully understood^[1,2]. It is now well established that close interactions exist between the luminal bacterial flora and the intestinal immune cells and tissues. Such interactions are involved in the onset and progression of several diseases including IBDs and colorectal cancer (CRCs)^[3-7]. Dysbiosis of gut microflora may represent the "primum movens" of the chronic

inflammation characterizing IBD and triggering the stimulation of the immune system^[8-13]. Dismicrobism can cause alterations in the immune response and can, in particular, lead to gut associated lymphoid tissue (GALT) activation^[14]. The terms dysbiosis and dismicrobism are being used interchangeably, however, dismicrobism is sometimes more used in reference to the condition evolving consequent to the administration of antibiotics. Specifically, the microbiological imbalance (dysbiosis) causes to a modification of intercellular tight junctions^[15] leading to an effective penetration of antigens responsible of the activation of GALT with consequent tissue damage^[16]. Olsen and Co-workers tested the reactivity of intestinal T cells derived from patients with CD, UC and undetermined colitis against some bacteria. The results showed that T lymphocytes isolated from CD patients had a high reactivity against *Mycobacterium avium* compared to patients affected by UC and undetermined colitis. Moreover, the isolated T cells produced inflammatory cytokines like interferon (IFN)- γ and IL-17^[17]. These data suggest a possible involvement of *Mycobacteria* in CD immunopathology. Other bacteria belonging to *Clostridia* group, *Fusobacterium*, *Mycobacterium*, adherent invasive *Escherichia coli* (*E. coli*), *Proteus mirabilis*, *Klebsiella pneumonia* and *Proteobacteria* such as *Helicobacter spp* were found over-represented in the IBD condition^[18,19]. Nowadays several studies focus on the possibility to control IBD through the modulation of the immune system by re-establishment of eubiosis through specific probiotic mixtures. The homeostasis of the immune system is very important and involves the equilibrium between pro-inflammatory and anti-inflammatory cytokines such as IL-10 and others. Mgl1^{-/-} mice with a DSS induced colitis were studied before and after probiotic treatment. The induction of colitis in wild type mice showed a less severe phenotype compared to Mgl1^{-/-} mice and a lowered expression of IL-10 in the mutant mice, leading to the evidence that Mgl1 is involved in the regulation of IL-10 production. The results showed that *Lactobacillus* and *Streptococcus* were able to bind to Mgl1 receptor, leading to the hypothesis that these bacterial strains may be able to induce an anti-inflammatory response^[20]. These findings highlight the importance of gut microbiota both in the triggering and in the modulation of immune system in the chronic inflammatory diseases of the gastrointestinal tract.

Diagnosis of dysbiosis

There is a relatively wide spectrum of diagnostic tests used for the diagnosis of dysbiosis. They cover all four patterns of dysbiosis by analysis of both stool and urine for respective markers. Reference laboratories specializing in the evaluation of dysbiosis offer a comprehensive testing of various aspects of digestion, absorption, microbiology, metabolic markers and immunology including fetal secretory IgA and sometimes calprotectin. In addition, in some cases of medical necessity, fecal analysis may also include other standard components such as stool culture, stool parasitology and fecal occult blood^[21,22].

ETIOLOGICAL LINK DYSIOSIS TO IBD AND CRC

The etiology of IBD is linked to three basic parameters: (1) genetic predisposition; (2) a dysregulated immune response; and (3) an altered reaction to gut microorganisms. It is also well established that the mechanism of intestinal inflammation, acute or chronic, involves a complex myriad of pathogens (bacteria, viruses or fungi), chemicals, radiation, dietary components and hormones among others^[1,11,23]. It also involves another complex series interactions between immune cells (T cells B cells, NK cells, mast cells, macrophages), interleukins, bradykinins, complement system factors, prostaglandins, tumour necrosis factors, histamines, hormones and activated neutrophils products like myeloperoxidase, radicals and oxydants^[1,23]. In some cases, the body's reaction is not enough to rid itself from etiological agents and infecting organisms. The reaction persists as a chronic inflammation which proliferates into multiple complications, escalating the damage, especially in genetically susceptible individuals. The big question is how could this chronic inflammation progress and express itself, and how could its complications lead to the formation of cancerous cells? It is well documented that patients with this condition are more prone to the development of malignancy and that the microbiota could sustain the chronicity of the inflammatory state in the presence of a dysregulated immune response^[3,6]. Such a persistent inflammation is believed to play a significant role in CRC development^[24-26]. Studies in this field reported underlying genetic, proteomic, and epigenetic changes in IBD's and CRC's. Individuals with IBD have mostly permissive rather than causative genes^[23].

In CD, an early discovery on chromosome 16 (*IBD 1* gene) led to the identification of 3 single nucleotide polymorphisms (2 missense, 1 frameshift) in the *NOD2* gene (now called *CARD 15* is a polymorphic gene involved in the innate immune system and has more than 60 variations, of which 3 play a role in 27% of patients with CD, primarily in patients with ileal disease. Another early genome-wide association study looked at Jewish and non-Jewish case-control cohorts and identified 2 single nucleotide polymorphisms in the *IL23R* gene, which encode 1 subunit of the interleukin-23 receptor protein. Actually, 21 new loci were identified with an increased risk of developing CD such as the genes *CCR6*, *IL12B*, *STAT3*, *JAK2*, *LRRK2*, *CDKAL1*, and *PTPN22*. Most of these genes are involved in signal transduction in immune function. There is also a strong support for IBD-susceptibility genes on chromosome 5p131.1 which is a gene desert but does modulate expression of the *PTGER4* gene. A murine *PTGER4* knockout model has also a significant susceptibility to severe colitis^[26,27].

In UC, however, the genetic predisposition is less than that of CD. Actually, there is a set of genetic susceptibilities that show significant overlap with CD. A recent genome-wide association study found a

previously unknown susceptibility locus at *ECM1* and showed several common risk loci between UC and CD. Genes that are a risk of both diseases appear to have an influence on the immune environment of the intestine^[28].

Further susceptibility loci for UC have been found on 1p36 and 12q15. The 1p36 single nucleotide polymorphism is located near the *PLA2G2E* gene; involved in releasing arachidonic acid from membrane phospholipids, which leads to other pro-inflammatory lipids. The first 12q15 signal is located near the interferon (*IFN*)- γ , interleukin (*IL*)-26, and *IL*-22 genes, whereas the second 12q15 signal is located in *IL*-26. These genes have roles in the immune response to pathogens as well as the tissue inflammation processes. It was also shown that genetic predisposition increases the risk for one form of IBD while decreasing that of the other^[27].

Chlorinated adducts of DNA and RNA, which are generated from reaction with MPO products, are associated with repetitive infiltration of neutrophils and macrophages to the site of inflammation. Furthermore, these chlorination products increased in amount as the inflammatory state lengthened. Importantly, infiltration by these innate immune cells was accompanied by cancer initiation and progression. Actually, the contributing factors to the onset of colon carcinoma were infiltration of phagocytes and macromolecular damage to DNA and RNA (chlorination) mediated by neutrophil activity. In cases of UC, *IL*-8, *Cl*-Tyr, *SAA*, *CRP*, procalcitonin, *G-CSF*, and tissue plasminogen activator have been identified, whereas for CD cases, procalcitonin, *SAA*, *Nitro*-Tyr, and *IP*-10 were identified as the most influential factors^[23].

Albumin is the most abundant chlorinated species found in serum; it is synthesized and released in the liver, indicating the liver as a possible site for innate cell activation in IBD patients, and therefore a possible source of the chlorination and nitration products observed in serum. This finding suggests that 5-*Cl*-dC may be a contributing factor in the initiation of colon carcinogenesis through either mutational or gene silencing mechanisms. In addition, two interesting proteins were identified from a proteomics study: Orosomucoid 2 ("1-acid glycoprotein 2) and Peptidylglycan recognition protein 2 (*PGRP2*, also named *N*-acetylmuramoyl-L-alanine amidase). Association of orosomucoid 2 with UC disease activity was not established, but it was associated with development of colorectal cancer^[23].

Concerning colorectal cancer, it has been reported for several decades that carcinomas mostly develop from adenomas and chronic inflammations. In 1988, Vogelstein described four specific mutations that accumulate during the progression of adenomas to carcinomas. These mutations seem to involve gatekeeper genes, which enable genetic or epigenetic instability and support tumor growth^[29].

Various factors have been shown to be responsible for the accumulation of mutations in CRC including inheritance and environmental factors (*e.g.*, composition

of diet, obesity, diabetes mellitus, smoking, alcohol consumption) as well, besides the fact that chronic inflammation sustained by microbial flora is regarded as an important risk factor for the development of cancer^[30].

On the other hand, IL-6 is regarded as an important tumor promoting factor in many types of human cancer including glioma, lymphoma, melanoma as well as breast, ovarian, pancreatic, prostate, renal and, colorectal cancer^[29,31].

Various studies have found an increased expression of IL-6 in patients with CRC, where IL-6 levels are elevated in the serum of patients and in tumor tissue itself. According to a review article by Knupfer and Preiss, IL-6 expression can be associated with tumor stage, size, metastasis and survival of patients with CRC^[32].

Many studies have revealed that IL-6 is an important regulator of IBD pathogenesis, mainly through its effect on immune cell function in the gut. IL-6 trans-signalling has been shown to activate T cells in the lamina propria of patients with IBD and induces resistance of these cells against apoptosis through upregulation of anti-apoptotic factors such as Bcl-2 and Bcl-xl. IL-6 is deemed to act as a link between chronic inflammation and tumor development due to its increased expression in IBD patients^[29].

Dysbiosis and diet

There is a myriad of factors, in addition to intestinal microbiota, that play a crucial role in the development of IBD and CRC. An altered microbiota, termed dysbiosis, could lead to altered immune functions and increased risk of disease^[33]. The influence of diet on the composition of the microbiota has been well documented during the initial colonization phase, postnatally and beyond, even in adult life^[34]. Several studies demonstrated that dietary factors have a dominating role in altering the microbial community resulting in biological changes to the host. Actually dietary changes could explain 57% of the total structural variation whereas genetic factors accounted for less than 12%^[35]. For example, the “Western” diet, which is high in sugar and fat, causes dysbiosis affecting both gastrointestinal (GI) metabolism and immune homeostasis^[30]. Vegetarianism, which is high in fibers, results in increased short chain fatty acid production by microbes leading to a decrease in intestinal pH and consequently preventing the growth of potentially pathogenic bacteria such as *E.coli* and members of the Enterobacteriaceae^[36]. In brief, it is becoming well documented that the microbiota could be modified through dietary factors to enrich beneficial microbes and prevent diseases associated with dysbiosis or promote such diseases like; IBDs metabolic diseases like diabetes, obesity as well as irritable bowel syndrome, celiac disease and also CRC. Diet-induced dysbiosis affects disease susceptibility^[37].

Understanding how extrinsic factors such as diet alter disease susceptibility through modification in the microbiota could shed light onto the function of

microbes in healthy and diseased individuals. A strong and well documented correlation exists between the composition of the gut microbiota and diet. In general, dietary change could explain most of the total structural variation in gut microbiota in metabolic syndrome repeats^[35].

Such microbial changes in the GI tract have profound effects on host inflammatory and metabolic responses. So far, it is not known for sure whether diet-induced dysbiosis is a transient or long-term event^[38]. This is a very dynamic area of research since dietary choices are proven to modify the ecology of intestinal microbiota which could increase susceptibility to disease like, IBD and CRC.

Dysbiosis in CRC

The next-generation sequencing and taxonomic studies, based on ribosomal 16S bacterial genes, give a clearer picture of the microorganisms inhabiting our intestine in physiological and pathological conditions, although a healthy core microbiota has not been clearly identified. The microbiota analysis on tissues and fecal materials has identified various microbial groups associated with CRC. Stool samples derived from CRC patients harbour a higher population of bacteria belonging to the group *Bacteroides-Prevotella* compared with normal controls^[24]. Another study showed an enrichment in the luminal compartment of CRC patients compared to controls of bacteria belonging to *Enterococcus*, *Escherichia*, *Shigella*, *Klebsiella*, *Streptococcus*, and *Peptostreptococcus*, and a depletion of bacteria belonging to *Lachnospiraceae* family (butyrate-producing bacteria)^[25,39]. CRC is a multi-step process that includes adenoma formation. Studies of microbiota on intermediate stages revealed an increased abundance of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* at the intestinal mucosal surface in patients with adenoma compared with non-adenoma subjects^[34]. Moreover, resected tissues from adenocarcinoma patients and adjacent non-malignant sites showed expansion of the phyla *Bacteroidetes* and reduction of *Firmicutes* in tumors compared with healthy subjects^[4]. Interestingly, through whole-genome and RNA-sequencing approaches two independent groups found a high prevalence of *Fusobacterium* (over-represented also in IBD) in colonic tissues of CRC patients compared with normal controls^[40,41].

Modulation of immune response in IBD

The intestinal microbiota is involved in aetiopathogenesis of autoimmune and allergic diseases and can also play a role in the development of CRC^[6]. Many cancers arise from sites of infection and chronic inflammation. The strongest association of chronic inflammation with malignant diseases is found in inflammatory bowel diseases of the colon with a lifetime incidence of 10% or more. Thus, patients with IBD have a higher risk of CRC occurrence^[1,42]. Both innate and adaptive immune responses have been involved in the pathogenesis of IBD. The first one involves particular receptors

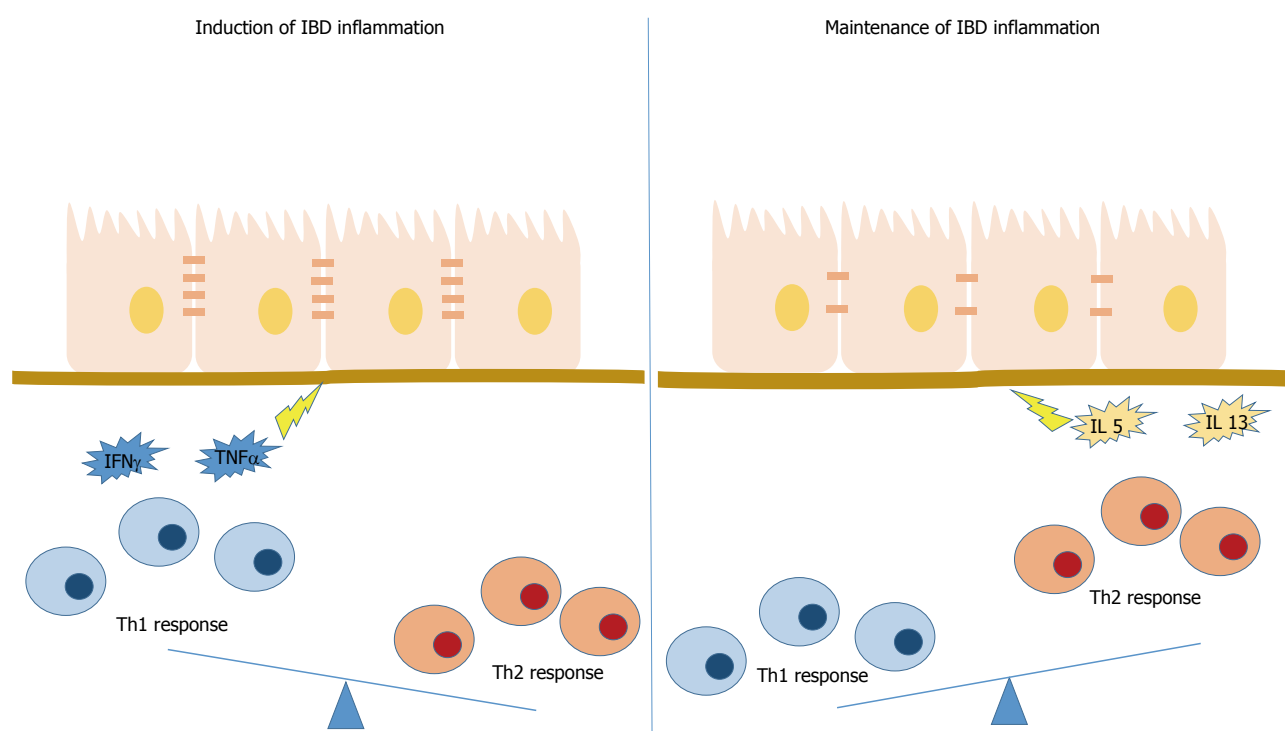


Figure 1 One hypothesis on the initiation of the inflammatory process in inflammatory bowel disease bases on an abnormal Th1 response mediated by the secretion of interferon gamma and tumor necrosis factor- α while the chronic inflammation is supported by interleukin-5 and interleukin-13 secreted by Th2 cells. IFN: Interferon; TNF: Tumor necrosis factor; IL: Interleukin; IBD: Inflammatory bowel disease.

called Toll like receptors or PRR (Pattern recognition receptors). It has been considered as an impairment of the innate immune response rather than an excessive activation, which may be involved in the development of CD. In fact some genes such as *NOD2* (an intracellular PRR involved in the recognition of the bacterial muramyl dipeptide) showed mutations consisting in a loss rather than a gain of function^[43]. Regarding the adaptive immune response, IBD is characterized by dysregulated T lymphocyte effector cells, and shows a Th1 and Th17 activation to the endogenous microbiota, and a lack of immune suppression typically afforded by Tregs^[44]. CD is considered a disease with an increased Th1 response while UC is a Th2-mediated disease. This paradigm relies on evidence showing that intestinal tissues derived from patients with active CD contain elevated levels of IL-12 and IL-18 (Th1-polarizing cytokines), compared with UC patients^[45,46]. Moreover, in CD patients, an increased amount of Th1 lymphocytes has been reported compared to control subjects^[45]. Evidence from studies conducted on humans and animal models suggest that the different subsets of lymphocytes play their role in different stages of IBDs^[43]. Studies of CD pathogenesis in SAMP1/YitFc mice indicate an involvement of Th1 cells in the initiation process of the disease. The establishment of chronic inflammation is supported by IL-5 and IL-13 cytokines, indicating a role of Th2 subset in this stage of CD^[47]. These data indicate that adaptive immune responses involved in IBD pathogenesis are more complex than the traditionally dichotomous Th1/Th2 paradigm (Figure 1).

Modulation of immune response in CRC

About 1%-2% of CRC patients have a pathological background consisting of intestinal mucosa inflammation^[48]. This “inflammatory background” of colonic mucosa can evolve to a less (low-grade) or more severe (high-grade) dysplasia, which through neoplastic transformation gives rise to carcinoma “*in situ*” and finally “*invasive*” carcinoma. Interestingly, many lines of evidence highlighted the importance of intestinal microbiota in the development of CRC^[42] and that the type of immune response generated by the gut commensal bacteria could potentially influence tumor immunity^[49]. Mice colonized with enterotoxigenic *B. Fragilis* exhibit colonic Th17 inflammatory infiltrates that are involved in induction of colon tumors, as indicated by inhibition of colon tumor formation following treatment with anti-IL-17 antibody^[50]. It is, therefore, possible that a microbiota favouring commensal bacteria, that induces a Th17 response, could have differential effects on tumors depending on the type of tumor or the stage of tumor development^[49]. The amount of lymphocytes infiltrated in the tumoral tissue is correlated to the prognosis of the patients affected by CRC^[51]. Among the different subsets of T lymphocytes studied in the immune system is the $\gamma\delta$ population. This lymphocytic subset is characterized by the presence of $\gamma\delta$ chains in their TCR and their representation in peripheral blood is < 10%. Since $\gamma\delta$ T cells express both natural killer receptors (NKG2D) and $\gamma\delta$ T cell receptors, they are considered as a link between innate and adaptive immunity^[52]. CRC comprises a small population of cancer stem cells (CSC) responsible for

tumor resistance to therapies. It has been observed that the treatment with the bisphosphonate zoledronate sensitizes colon CSCs to $\gamma\delta$ T cell cytotoxicity through the production of pro-inflammatory cytokines [IFN γ and tumor necrosis factor (TNF)- α] and granule exocytosis pathway. The $\gamma\delta$ T cells response is highly dependent on isoprenoid production by tumor cells^[53]. Isoprenoids are a class of natural compounds derived from five-carbon isoprene units assembled and modified in different ways. Most of them have multicyclic structures found in all classes of living organisms such as bacteria. These compounds are under investigation for their involvement in bacterial metabolism and immunomodulation of $\gamma\delta$ T lymphocytes. Isoprenoids are synthesized through the mevalonate pathway or the alternative 2C-methyl-D-erythritol 4-phosphate (MEP) pathway. It has been observed that the MEP intermediate pathway 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate (HMB-PP) can activate human $\gamma\delta$ T cells. Some pathogen bacteria such as *Campylobacter jejuni* and *Salmonella enterica* have genes involved in the MEP pathway. Since HMB-PP and other isoprenoids are able to induce activation of $\gamma\delta$ T cells, this metabolic feature shared by bacteria and host cells should be used to fight together dysbiosis and the tumorigenesis process of CRC.

Modulation of Heat shock proteins by gut microflora

The “chaperoning system” is a concept that was proposed in 2008^[54]. Heat shock proteins (Hsps) are cellular complexes involved in processes like folding, refolding, translocation and degradation of intracellular proteins under normal and stress conditions^[6,55]. Hsp chaperones are also involved in many cellular processes and interact with the immune system. They start to be considered as potential biomarkers of carcinogenesis, correlating their expression to the degree of differentiation and aggressiveness of certain tumors. Pathological conditions in which chaperones become etiological factors are called “chaperonopathies” and are classified into three categories: by defect, by excess, and by mistake^[56]. Commensal microbiota may affect the immune system influencing the expression of specific Hsps. In particular, Hsp60 and Hsp70, both constitute a group of auto-antigens able to trigger immune-regulatory pathways, involved also in other human inflammatory processes such as rheumatoid arthritis^[57], Type 1 diabetes mellitus^[58], atherosclerosis^[59] and allergy^[60], through the stimulation of both innate and adaptive immune responses^[61]. These proteins were found increased in both tissue and serum of patients with UC^[7]. The microbiota is able to regulate Hsp expression. Studies conducted on patients affected by IBD showed a decrease of Hsp60, Hsp10 and Hsp70 in epithelium and lamina propria after a combined therapy of 5- amino salicylic acid plus probiotics (*Lactobacillus Salivarius*, *Lactobacillus Acidophilus*, and *Bifidobacterium Bifidum*)^[62]. Treatment of gut epithelial cells with *Lactobacillus GG* showed an increase in Hsp25 and Hsp72 (considered as cytoprotective Hsps) in a time

and concentration dependent manner. These data suggest that the microbiota is able to influence Hsps expression in colocytes.

Double role of microbiota: Prevention or promotion of carcinogenesis

Gut microflora has a great importance; it influences the colonocyte's life under multiple aspects. In certain groups of individuals, the onset of CRC is strongly related to diet and composition of the microbiota^[42]. The aforementioned correlation could be explained through the anticancer effects of bioproducts of microflora metabolism^[63]. Small-chain fatty acids (SCFAs), particularly butyrate, formed from the bacterial fermentation of indigestible carbohydrates, are nutrients and growth signals for the intestinal epithelium and may play a role in CRC prevention^[64]. In normal colonocytes, butyrate prevents apoptosis and subsequent mucosal atrophy^[10,65]. In contrast, in colon carcinoma cells, butyrate exerts a protective role against the onset of colonic cancer stimulating differentiation, inhibiting cell proliferation, inducing apoptosis and inhibiting angiogenesis^[66]. The role of butyrate resides in its effects on enzymatic functions such as the inhibition of the histone deacetylase. This enzyme is responsible of the maintenance of the acetylated state of the histones. Acetylation is important for the access to the chromatin by the enzymes responsible of the transcription of important genes such as those involved in the inhibition of tumorigenesis (*p21/Waf1*)^[67]. Inhibition of histone deacetyltransferases enzymes (HDAC) is one of the therapeutic approaches used in anticancer therapy. Microbial metabolism of cruciferous vegetables or garlic leads to the production of compounds such as sulforaphane N-acetyl-cysteine, allyl mercaptan and butyrate. Thus, microbe-derived metabolites can counteract the carcinogenetic process by triggering cell cycle arrest and apoptosis of tumoral cells through interference with HDAC activity^[10,68]. Endoluminal accumulation of toxic compounds can exert a mutagenic action on intestinal mucosa. The microbial fermentation of charred meat leads to the production of poliheterocyclic amines (PAH). These compounds are able to damage the DNA of colonocytes. Bacteria belonging to the *Clostridium* species are able to convert the bile acids into secondary products like deoxycholic acid (DCA). This compound is capable to induce the carcinogenesis process^[69]. Tumorigenesis is a multi-step process in which the damage in the DNA is the essential pre-condition for tumoral induction. *E. coli* has a genic cluster called Pks that encodes for a toxin called calibactin. This was illustrated in studies conducted in mice which are not able to produce IL-10. They were treated either with one strain of *E. coli* in which Pks was deleted and the others with the wild type strain. The results of this experiment showed that the genic cluster Pks was only able to induce damage on the DNA (assessed through the presence of H2AX marker) but not sufficient to induce

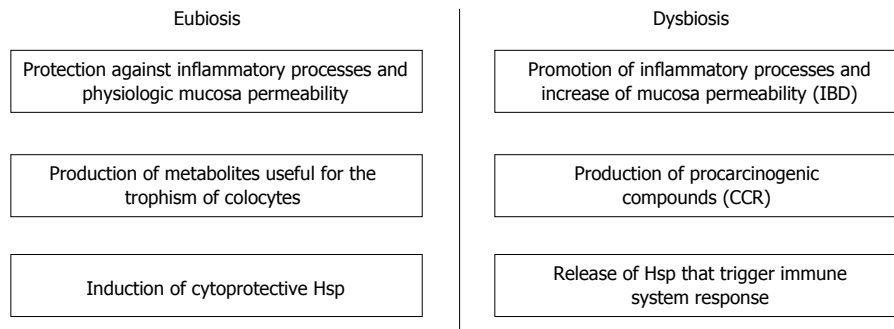


Figure 2 Eubiosis and dysbiosis exert different biochemical effects in the context of colocytes pathophysiology. IBD: Inflammatory bowel disease.

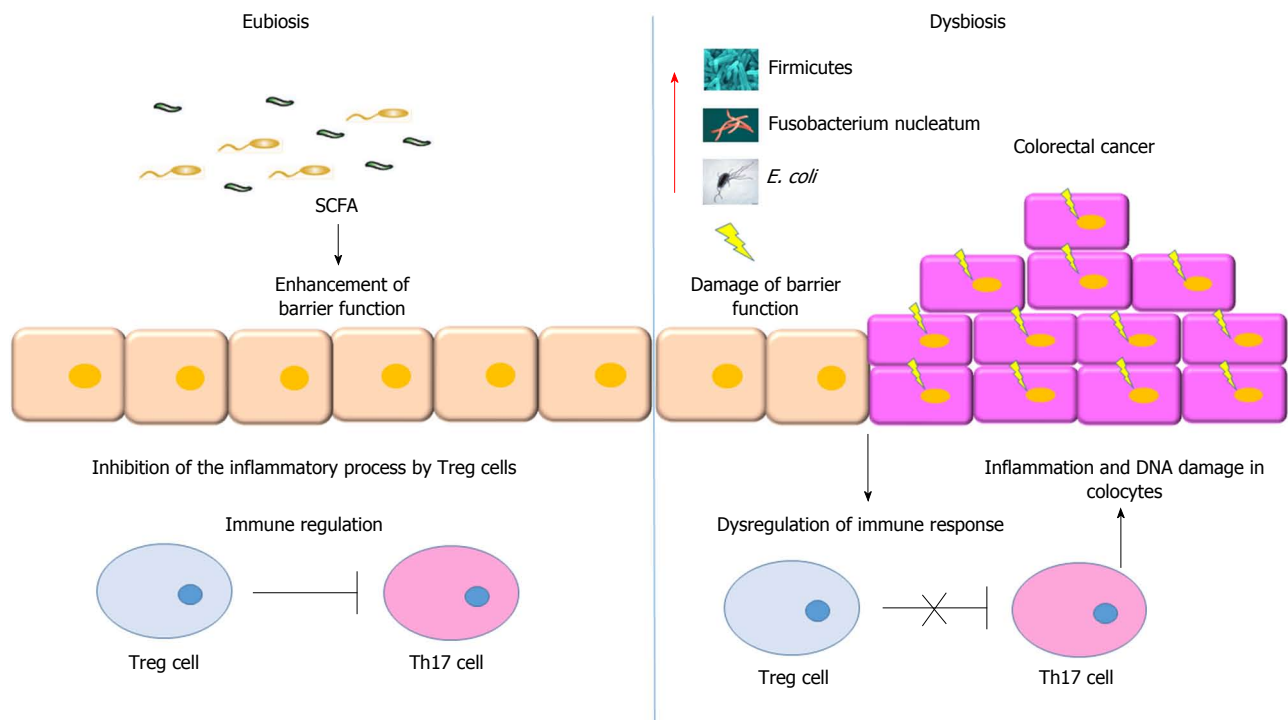


Figure 3 At eubiosis stage, the intestinal epithelium hosts a rich and equilibrated microbiota that promotes the barrier function. Microbial dysbiosis favors the production of genotoxins and metabolites associated with carcinogenesis. Moreover dysbiosis induces dysregulation of immune responses that cause inflammation promotion of carcinogenesis. SCFA: Small-chain fatty acids; *E. coli*: *Escherichia coli*.

rectal carcinogenesis^[65,70]. Experimental data show that consumption of omega-3 polyunsaturated fatty acids is able to decrease the incidence of sporadic colorectal cancer. Eicosapentaenoic free fatty acid reduces polyp formation and growth in models of familial adenomatous polyposis^[71]. The intake of docosahexaenoic acid can modify the gut microflora. Breast milk is rich in omega-3 fatty acids. Some studies showed that *Bifidobacteria* are more represented in breastfed infants rather than in formula-fed infants. In another study, it was shown that the supplementation with fish oil is able to determine changes in *Bacterioidetes* among children 9-18 mo old^[72]. An understanding of the comprehension of the correct equilibrium in the prevalence of the different bacterial strains composing human microbiota may represent one of the keys for the better understanding of CRC etiopathogenesis. In fact, bacterial metabolism has a high impact on the biochemistry of colocytes (Figure 2). This field of study may open the road to therapies oriented

not only towards specific probiotic-based therapies but also to targeted “prebiotic-based” approaches for the prevention or treatment of CRC.

CONCLUSION

The role of environmental factors involved in CRC aetiopathogenesis remains to be fully elucidated. The qualitative and quantitative modifications of the microbiota can make people more susceptible to develop IBD. In fact, these changes can trigger the production of inflammatory mediators in the mucosa that, in a large temporal window, can also exert a pro-carcinogenic role. The immunological and biochemical evidences related to dysbiosis allow the global comprehension of the background on which relies the development of CRC (Figure 3). These findings suggest a close connection between dysbiosis, IBD and CRC. Up till now, the direct or indirect role of bacteria in the induction of genomic

damages in colocytes is not fully confirmed. Several clinical trials have focused the preventive and therapeutic action of probiotics in the treatment of digestive diseases. Probiotics could inhibit the inflammatory and the carcinogenic processes through several pathways including the re-equilibration of the host immune responses and through the anti-proliferative activity on tumor cells. However, further clinical studies seem necessary to clarify the complex interplay between dysbiosis, inflammation and CRC.

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Role of interleukin-22 in inflammatory bowel disease

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in the pathogenesis of IBD, as well as its therapeutic potential.

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Key words: Inflammatory bowel disease; Interleukin-22; Signal transducer and activator of transcription 3

Core tip: Interleukin (IL)-22 is expressed by adaptive immune system cells and innate lymphocytes. Several cytokines and many transcriptional factors and T regulatory cells can regulate IL-22 expression. Through activation of signal transducer and activator of transcription 3 signaling cascades, IL-22 induces antimicrobial, proliferative and antiapoptotic pathways, which can help fix damaged tissue and promote tissue repair mechanisms. IL-22 is also associated with inflammatory bowel disease (IBD) susceptibility genes that regulate inflammatory responses in tissues. All of these processes play crucial roles in IBD pathogenesis and collectively provide an important rationale for the development of novel therapeutic measures for this disease.

Abstract

Inflammatory bowel disease (IBD) is a chronic inflammatory disease thought to be mediated by the microbiota of the intestinal lumen and inappropriate immune responses. Aberrant immune responses can cause secretion of harmful cytokines that destroy the epithelium of the gastrointestinal tract, leading to further inflammation. Interleukin (IL)-22 is a member of the IL-10 family of cytokines that was recently discovered to be mainly produced by both adaptive and innate immune cells. Several cytokines and many of the transcriptional factors and T regulatory cells are known to regulate IL-22 expression through activation of signal transducer and activator of transcription 3 signaling cascades. This cytokine induces antimicrobial molecules and proliferative and antiapoptotic pathways, which help prevent tissue damage and aid in its repair. All of these processes play a beneficial role in IBD by enhancing intestinal barrier integrity and epithelial innate immunity. In this review, we discuss recent progress in the involvement of IL-22

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the small intestine and colon, and includes Crohn's disease (CD) and ulcerative colitis (UC). Despite extensive research efforts, however, the etiology of IBD remains unclear. The current opinion about IBD pathogenesis is that the disease results from interactions between environmental factors, mainly microbes of the intestinal lumen and their products,

and dysregulation of immune responses in genetically susceptible individuals^[1]. Certain harsh environments that may affect barrier integrity (to increase barrier permeability to luminal macromolecular substances, such as protein antigens and microbial products) and over-absorption of luminal microbial products (which has been ascribed to a number of mucosal pathologies) can lead to an over-activation of immune system, thus resulting in mucosal inflammation^[2].

Interleukin (IL)-22, a member of the IL-10 cytokine family which is composed of IL-10, IL-19, IL-20, IL-24 and IL-26^[3], is expressed by both the cells of the innate immune system [such as dendritic cells (DCs), lymphoid tissue inducer (LTi)-like cells and natural killer (NK) cells] as well as on the surface of adaptive lymphocytes (including CD4⁺ T cell subsets, CD8⁺ T cells and so on)^[4]. Several cytokines [such as IL-23, IL-6, tumor necrosis factor (TNF) α , IL-1 β , transforming growth factor (TGF) β and IL-17], many of the transcriptional factors (signal transducer and activator of transcription (STAT) 3, RAR-related orphan receptor (ROR) γ t and aryl hydrocarbon receptor (AhR)]^[5] and T regulatory cells (Tregs) are known for their regulation of IL-22 expression^[6]. Through activation of the Jak-STAT signal transduction pathway, IL-22 induces proliferative and anti-apoptotic pathways, as well as the production of antimicrobial peptides, which help prevent tissue destruction and assist in its repair and restoration^[7]. IL-22 is also associated with IBD susceptibility genes that are crucial for regulating tissue responses during inflammation^[8]. All of these processes play critical roles in the pathogenesis of IBD. In recent years, it was demonstrated that treatment with recombinant cytokine or gene therapy involving IL-22 can suppress the inflammatory response and alleviate tissue injury^[8,9]. Thus, these findings suggest that further research focused on IL-22 may elucidate the underlying mechanisms of IBD and facilitate the development of novel effective, targeted therapeutic approaches for IBD. This review focuses on IL-22 and its functional role in IBD.

IL-22

IL-22 signaling

The IL-22 receptor is a heterodimer composed of IL-22 receptor 1 (IL-22R1) and IL-10 receptor 2 (IL-10R2)^[10]. IL-10R2 is ubiquitously expressed by most cell types, while the expression of IL-22R1 is limited to nonhematopoietic cells (such as hepatic cells, pancreatic cells, kidney cells, epithelial cells, and skin keratinocytes)^[10,11]. Therefore, the expression profile of IL-22R1 determines how IL-22 specifically targets innate cell populations, and not adaptive immune cells^[12].

STAT3, STAT1 (in a relatively small number of cells) and STAT5 (in certain cells) were shown to be activated after IL-22 stimulation^[13]. Further analysis has also demonstrated that IL-22 signaling propagates downstream phosphorylation signals, including several

of the mitogen-activated protein kinase (MAPK) pathways (extracellular signal-regulated kinase (ERK)1/2, MEK1/2, C-Jun N-terminal kinase (JNK), and p38 kinase), and STAT1, STAT3 and STAT5 by utilizing Janus kinase (JAK)1 and tyrosine protein kinase (TYK)2^[14] (Figure 1). The capacity of IL-22 to activate JNK, ERK1/2 and p38 MAPK pathways has been implicated in liver diseases^[14]. Moreover, the strong activation of IL-22 to stimulate STAT3 has been confirmed in human colon cancer cell lines, human colonic biopsy, as well as the primary mouse colonic epithelial cells^[15,16]. In fact, a recent study has shown that, compared with IL-6, IL-22 has a stronger ability to activate STAT3^[17]. Pickert *et al*^[18] have demonstrated that in dextran sulfate sodium (DSS)-induced colitis, the activation of epithelial STAT3 is more dependent on IL-22 than on IL-6, a well known activator of STAT3. This is due to IL-22R1 utilizing its constituent C-terminal tail to interact with the coiled-coil domain of STAT3, which has been to conformed in a recent discovery as a novel mechanism to activate STAT3^[19]. Similar to other IL-10 family cytokines, IL-22 primarily relies on STAT3 to mediate its functions. Binding of cytokines to this receptor results in the activation of STAT3 signaling pathways, which in turn leads to the induction and production of various tissue-specific genes, including serum amyloid A (SAA), antimicrobial proteins (β -defensin, Reg3c and lipocalin-2) and mucins. Meanwhile, IL-22 also induces proliferative and antiapoptotic pathways in some responsive cells of certain tissues^[10,20].

Cellular sources of IL-22

Basu *et al*^[21] have suggested that both innate lymphoid cells (ILCs) and T cells produce IL-22. They showed that IL-22 produced by ILCs was strictly IL-23-dependent, and that the development of IL-22 induced by CD4⁺ T cells was *via* an IL-6-dependent mechanism that was augmented by IL-23 and was dependent on both transcription factors T-bet and AhR. At the same time, Wolk *et al*^[22] confirmed that activation of murine T cells, especially T helper (Th) 1 cells, mainly express IL-22. A novel Th subset - the Th17 cells - was identified in 2005^[23,24]. IL-17 (or IL-17A), a hallmark cytokine preferentially expressed by Th17 cells, distinguishes these cells from other Th subsets, such as Tregs, Th1 and Th2 cells^[25]. Th17 cells play an essential role in host defense, especially against extracellular bacteria and other infectious bacteria, and are involved in the pathogenesis of various autoimmune diseases^[26,27].

The level of IL-22 produced by Th17 cells is much higher than that of the production from undifferentiated Th0 cells or Th1 cells. However, the expression and regulation of IL-22 and IL-17 produced in T cells are unparalleled. Researchers have discovered that IL-6 and TGF β are both required for inducing IL-17 expression in naïve T cells, yet IL-6 alone can sufficiently promote the expression of IL-22^[28-30]. In fact, TGF β has been shown to suppresses IL-22 production in a dose-

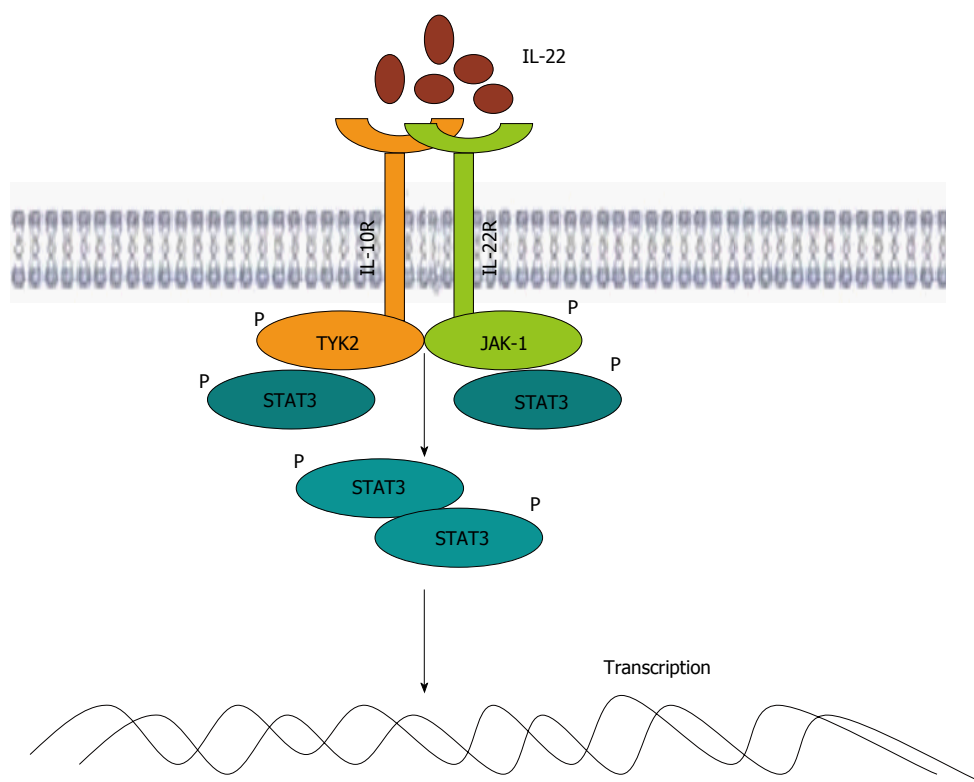


Figure 1 Interleukin-22 signaling pathway. IL-22 binds to a heterodimeric receptor composed of IL-10R2 and IL-22R1, activating JAK1 and TYK2, which self-phosphorylate, resulting in binding and phosphorylation of STAT3. Then, STAT3 translocates to the nucleus and induces target genes. IL: Interleukin; JAK: Janus kinase; TYK: Tyrosine protein kinase; STAT: Signal transducer and activator of transcription.

dependent manner^[28]. Through activation by anti-CD3 or concanavalin A (ConA), human T cells can produce IL-22^[31]. Based on studies using the lineage marker chemokine CCR receptor (CCR) 6 and CCR4, human Th17 cells produced *in vitro* or purified *ex vivo* from blood were shown to preferentially express IL-22^[32,33]. Moreover, IL-17- and IL-22-expressing cells in human peripheral blood mononuclear cells (PBMCs) are defined by another surface maker, CD161^[34]. The above-mentioned Th17 cells can produce IL-22 and IL-17; in addition, CD161⁺ human CD8⁺ T cells can also generate these two cytokines^[35].

Recent studies have also demonstrated a unique cell subset, designated as the IL-22-producing CD4⁺ Th subset, in human peripheral blood, which expresses neither IL-17 nor interferon (IFN)- γ ^[36-38]. In skin, these cells mainly express CCR10. Moreover, the human IL-22-producing T cells can also be generated from naïve CD4⁺ T cells in the presence of IL-6, rather than TGF β , which is consistent with what has been reported in the mouse system^[36]. Human Langerhans cells are able to differentiate T cells into the only IL-22-producing Th cells *in vitro*^[39].

The human innate immune cell types, such as NK and LTi cells, can also produce IL-22^[40,41]. In addition to CD4⁺ T cells, the Th17 cells, CD8⁺ T cells and NK T cells also express high levels of IL-22 upon activation, especially when activation occurs along with IL-23 intervention^[28,42]. Recently, LTi cells and developmentally-

related NK-like cells (NK22), which express the NK marker NKp46, were demonstrated to be the main innate sources of IL-22 expression, especially in the intestinal tract^[43,44]. Treatment of NK cells with IL-2 and IL-12 was shown to lead to expression of IL-22^[45]. Human immature NK cells, defined as CD161⁺CD117⁺CD34⁻CD94⁻ cells, express both IL-22 and AhR^[46]. The equivalent NKp46⁺ NK-like cells in mice have been found to be developmentally linked to LTi cells^[47,48].

Finally, subsets of myeloid cells express the IL-23 receptor (IL-23R) and combine with IL-23 to release lower levels of IL-22^[49,50]; those cells that produce high levels of IL-22 may be the major cells of IL-22 origin in mucosal immunity. In contrast to the IL-22 produced by leukocytes, such IL-22 targets mainly tissue epithelial cells rather than immune cells^[51]. Although expression of IL-10R2 is widespread, IL-22R1 expression has only been detected on epithelial cells. Upon binding to its receptors on the surface of these epithelial cells, IL-22 produces an accelerating effect on the proliferation and differentiation of these cells, and induces these cells to express genes involved in host defense and wound-healing responses^[52].

These cellular functions of IL-22 underlie its crucial role in epithelial barrier defense, especially against invading extracellular bacteria. In fact, in a preclinical model of mucosal immune responses to Gram-negative bacteria, such as *Klebsiella pneumoniae* and *Citrobacter rodentium*, IL-22 played an indispensable role^[53]. Moreover, IL-22 is associated with the development of various

human autoimmune diseases^[25]. The expression of IL-22 is unregulated in autoimmune diseases, such as IBD, rheumatoid arthritis and psoriasis. IL-23 appears to be a principal inducer of IL-22 in Th17, NK or NK-like cells, suggesting that IL-22 acts a pivotal mediator in IL-23-dependent immune reactions in skin and mucosal epithelia by stimulating innate antimicrobial responses as well as promoting tissue repair^[54].

Regulation of IL-22 expression

IL-23 is a member of the IL-12 cytokine family, and its stimulation of activated T cells induces IL-22 expression^[54]. Research has found that *il23a*^{-/-} and *il22*^{-/-} mice are both highly susceptible to infection with extracellular Gram-negative bacteria, suggesting that a critical function of IL-23 in infection is to induce IL-22 expression^[55,56]. Additionally, IL-23 has been found to be important in the terminal differentiation of Th17 cells, assisting in their proliferation and effector functions^[56]. Therefore, the ability of IL-23 to enhance Th17 cell proliferation appears to be linked to IL-22 expression.

In addition to IL-23, other cytokines have been found to regulate the expression of IL-22. In cultures of purified naïve murine CD4⁺ T cells, IL-6 and T cell receptor (TCR) stimulation, or IL-6, TNF α , IL-1 β and TCR stimulation, was sufficient to induce IL-22 expression^[57]. Increasing concentrations of TGF β dose-dependently inhibited IL-22 expression while maintaining stable IL-17A expression. It has recently been demonstrated that IL-17A can partially inhibit the expression of IL-22 from Th17 cells *in vitro* and *in vivo*, indicating that Th17 cell-associated IL-17A can also negatively regulate IL-22 expression. IL-22 expression in $\gamma\delta$ T cells can also be induced independently of IL-23 and TCR stimulation by IL-1 β , as well as Toll-like receptor (TLR) 1, TLR2, and dectin-1 ligands^[54,58].

Similar to cytokine-mediated regulation of IL-22, many of the transcriptional factors are known for regulation of IL-22 expression. STAT3 is critically involved in the induction of IL-22 expression in T cells^[59]. Similarly, ROR γ t, a lineage-specifying transcription factor for the differentiation of Th17 cells, is also required for optimal expression of IL-22. STAT3 and ROR γ t both control expression of IL-23R, and this regulation may account for their ability to promote IL-22 production in Th17 cells. Therefore, many of the same transcription factors involved in Th17 cell differentiation are also required for IL-22 expression in CD4⁺ T cells^[60]. In addition, AhR is a ligand-dependent transcription factor that is best known for its role in mediating toxicity to the organic compound dioxin. AhR also partially contributes to the differentiation of Th17 cells and is required for expression of IL-22, thus linking IL-22 and Th17 cells to toxicity following exposure to different environmental compounds^[61]. A number of IL-22-producing innate cell populations have also been found to express STAT3, ROR γ t and AhR, yet the involvement of these transcription factors in regulating IL-22 expression

in innate cell populations has yet to be examined^[62] (Figure 2).

Recent studies have demonstrated a close relationship between CD4⁺Foxp3⁺ Tregs and proinflammatory IL-17-producing Th17 cells expressing the lineage-specific transcription factor ROR γ t. It has been shown that IL-17-secreting Foxp3⁺ T cells that express ROR γ t share features of conventional ROR γ t⁺ Th17 cells. However, ROR γ t⁺Foxp3⁺ Tregs mostly fail to secrete IL-22 after phorbol 12-myristate 13-acetate/ionomycin stimulation^[63]. Foxp3 transcription factor binding sites (TFBSs) in the IL-22 promoter restrain ROR γ t⁺Foxp3⁺ T cells to produce IL-22 at the transcriptional level^[64]. Despite the decreased expression of IL-22 in Foxp3⁺ Tregs, it has been found that Tregs can promote naïve T cell differentiation. In a mouse model of infection with oral *Candida albicans*, Foxp3⁺ Tregs were shown to powerfully promote the transition of naïve CD4⁺ T cells to responding CD4⁺ cells (Tresps)^[65]. Tresps markedly produce IL-22. Therefore, there is the possibility that Tregs can regulate the expression of IL-22.

IL-22 IN PATHOGENESIS OF IBD

IL-22 regulates intestinal barrier immunity

The IL-22 signaling pathway is activated through a heterogeneous receptor complex composed of two subunits, IL-22R1 and IL-10R2^[66]. Although IL-10R2 is widely expressed on almost all of the cell types, the expression of IL-22R1 is restricted to the surfaces of nonhematopoietic cells such as epithelial cells, hepatocytes and keratinocytes^[67]. This limited expression of IL-22R1 on nonhematopoietic cells allows IL-22 to specifically target innate cell populations within such tissues as the skin, kidney, digestive tract and respiratory systems^[68]. A wide variety of innate and adaptive immune cells, including CD4⁺ T cells, and most notably Th17 and Th22 cells, CD8⁺ T cells, LTi cells, NK cells and DCs, can produce IL-22^[69]. Upon binding to the IL-22R1 and IL-10R2 receptor complex, these cells produce IL-22 to activate receptor-associated JAK1 and TYK2, resulting in tyrosine phosphorylation of STAT3^[70]. This in turn allows IL-22 to induce different kinds of tissue-specific genes, including those encoding proteins involved in antimicrobial defense, cellular differentiation, and expression of mucins; a large, heavily glycosylated family of proteins in the gastrointestinal tract forms a protective layer, which serves to separate commensal bacteria from pathogenic bacteria in the epithelium layer, thereby minimizing the immune response^[71]. Through the production of antimicrobial peptides, enhancement of epithelial regeneration, and regulation of wound healing, IL-22 plays a particularly vital role in regulating intestinal inflammatory responses^[72]. Furthermore, a direct effect of IL-22 on colonic epithelium is proliferation of epithelial cells, which maintains the integrity of the intestinal epithelium.

Recent studies have focused on possible protective

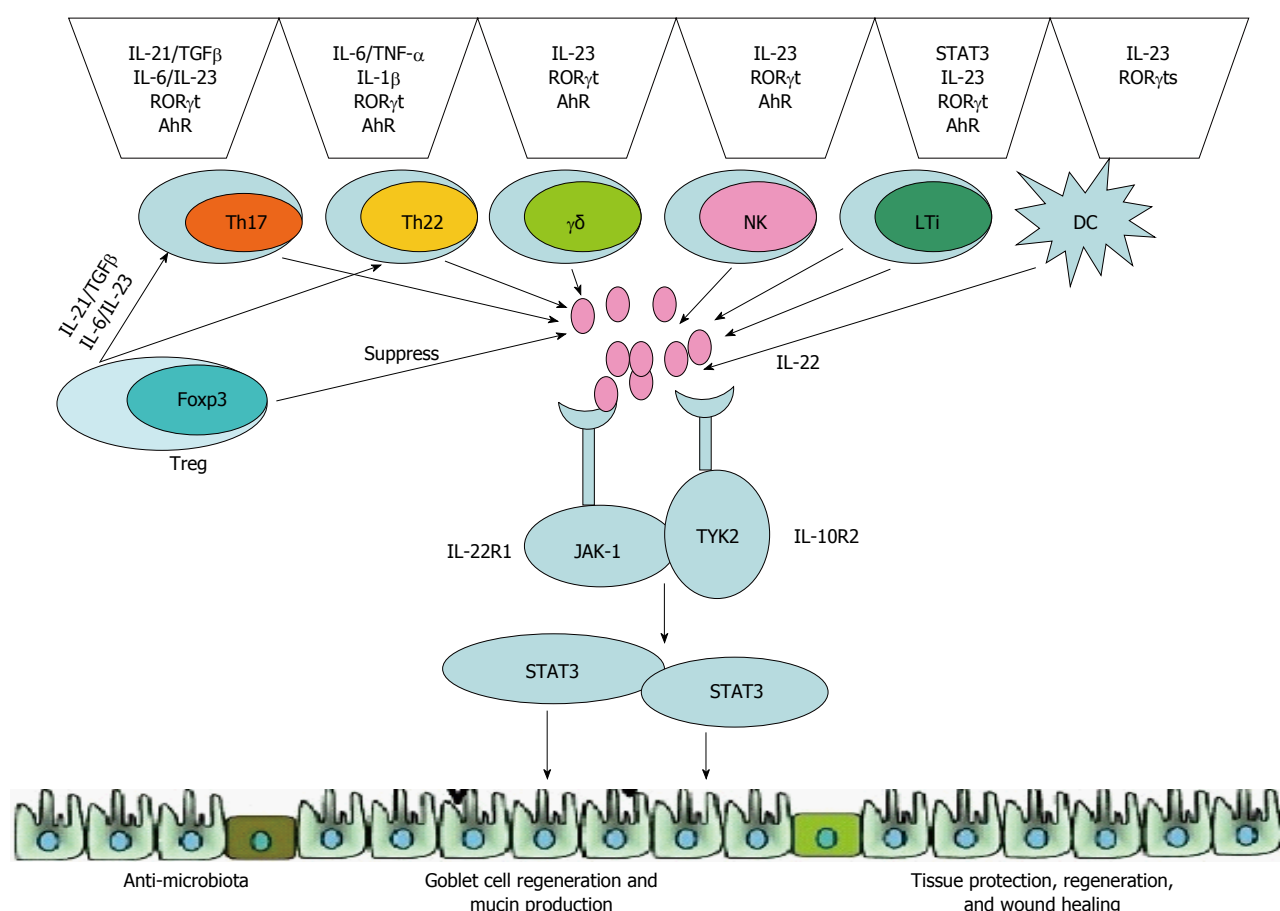


Figure 2 Protective function of interleukin-22 in inflammatory bowel disease. In the intestine, innate and adaptive immune cells, cytokines and many of the transcriptional factors can induce and regulate expression of IL-22. Through activation of STAT3 signaling cascades, IL-22 induces antimicrobial molecules and proliferative and antiapoptotic pathways, and helps regenerate goblet cells and produce mucin proteins, which could prevent tissue damage and aid in its repair. IL: Interleukin; STAT: Signal transducer and activator of transcription.

effects of IL-22 in IBD, and have used several DSS-induced as well as Th1- and Th2-mediated colitis mouse models^[73,74]. In the DSS-induced colitis model, feeding mice DSS causes disruption of the intestinal epithelial barrier, leading to colitis within 1 wk. In IL-22 knockout mice or wild-type (WT) mice, administration of neutralizing anti-IL-22 antibodies leads to more extensive epithelial destruction and inflammation in the colon, more severe weight loss, and more impaired recovery compared to the DSS-induced acute colitis model. In addition, T cells from IL-22^{-/-} mice or IL-22-deficient mice cause a more severe colitis in the T cell transfer model of IBD^[75]. In a Th1-cytokine-mediated model of colitis, expression of IL-22 by CD4⁺ T cells is crucial for relief of disease severity. Sugimoto *et al*^[76] showed that receipt of supplemental IL-22 leads to rapid amelioration of local intestinal inflammation in the colons of Th2-mediated chronic colitis. IL-22 knockout mice showed delayed recovery from DSS-induced acute colonic injury. Treatment with neutralizing anti-IL-22 antibodies also impaired the recovery of WT mice. Finally, in IL-22-deficient and RAG1-deficient double knockout mice, lacking both T and B cells, no recovery was observed^[77].

IL-22 gene delivery mediates STAT3 activation

specifically within colonic epithelial cells and enhances reconstitution of goblet cells and production of mucus, thereby reinforcing the mucus barrier function within the gastroenterology tract^[78]. In DSS-induced acute colonic injury, recovery is significantly impaired and delayed in IL-23R-deficient and RAG2-deficient double knockout mice lacking of IL-22 expression, and treatment with recombinant IL-22 rescues the recovery in these mice^[79]. Pancreatic cells produce TGFβ and IL-10 upon IL-22 stimulation, which can inhibit IFN-γ production, facilitating relief of intestinal injury. These mouse models of colitis suggest that IL-22 plays a protective role in IBD through its ability to improve the integrity of the mucosal barrier and enhance the inherent epithelial defense function.

IL-22 responses to intestinal pathogens

In addition to maintaining the mucosal barrier function in the gastrointestinal tract, IL-22 induces genes to encode anti-microbial proteins involved in bacterial defense and protection of intestinal mucosa, suggesting a role for IL-22 against extracellular bacteria in the innate immune system. CD and UC are thought to be driven by an abnormal immune response to the intestinal

flora^[80]. However, since intestinal dysbacteriosis is also a characteristic of IBD pathogenesis, it is difficult to determine whether there is an inflammatory response to abnormal flora or if an abnormal inflammatory response is altering the microbial communities^[81]. Intestinal flora, as an environmental factor, may be associated with genetic susceptibility that alters the interactions between ourselves and our microbiome. The first major susceptibility gene discovered for CD is NOD2 (or CARD15), which is known as a receptor for bacterial peptidoglycan (PGN)^[82]; another susceptibility gene, ATG16L1, has been shown to be critical for autophagy^[83]. The intestinal flora may also lead to disorders of intestinal lymphoid cell subsets, such as Th17 cells and innate lymphocytes, which are important for regulating mucosal immunity^[83]. Although there have been numerous studies investigating stool samples of and mucosa-associated bacteria in IBD patients, there has been a lack of consensus between the associations observed in these studies^[83].

Although extensive changes have been reported, such as expansion of the *Proteobacteria* phylum in IBD patients^[84], only few specific associations have been reproducibly identified. Although the causes of changes in microbiota species that can trigger IBD remain unclear, and studies on this subject are continuing, the general theme observed so far is that the diversity of microbial communities is significantly decreased in IBD^[85]. There have also been repeated observations of the microbiota composition being disrupted during inflammation, resulting in dysbiosis that may induce or perpetuate the inflammatory condition. However, both host genotype and the environment have major impacts on the shape of such dysbiosis, as well as upon which members of the microbiota can stimulate pathogenic immune responses^[86].

By promoting the maintenance of intestinal epithelial barrier function, IL-22 can prevent the spread of pathogenic microorganisms in the gut, such as enteropathogens, including *Citrobacter rodentium* and *Salmonella typhimurium* (enteric ecotype) in the gastrointestinal tract, thereby limiting bacterial growth. Tregs promote IL-22-dependent clearance of fungi during acute *Candida albicans* infection^[87,88]. In addition, IL-22 can help to eliminate pathogenic microorganisms by inducing various anti-microbial proteins (Figure 2). IL-22 has already been confirmed as a regulator of the expression of antimicrobial proteins such as the S100 family proteins (S100A7, S100A8 and S100A9), β -defensin family proteins (β -defensins BD2 and BD3), Reg family proteins (RegIIIa, RegIIIb and RegIIIc) and lipocalin-2^[83,89-91]. These proteins may be important in the control of gut pathogens. IL-22 plays a protective role in the host inflammatory response to microbial infections or in promoting the release of inflammatory mediators, depending on the type of pathogenic microorganisms causing the infection.

Song *et al.*^[92] showed that IL-22 plays an crucial role in host defense immunity against infection with the

Gram-negative enteric bacteria *Citrobacter rodentium*, as an inducer of the expression of antibacterial peptides in colonic epithelial cells. The protective effect of IL-22 in systemic infections caused by *Salmonella enterica* has been demonstrated. IL-23-dependent IL-22 was required for both liver cells' survival and pathogen defense against systemic *Salmonella* infection in mice, especially when accompanied by decreased production of IL-12^[93].

IL-22 is not only able to protect our intestine against bacterial pathogens, but also plays a protective role in intestinal fungal infections with *Candida albicans*. Compared with infected WT mice, IL-22 knockout mice infected with *Candida albicans* hyphae intragastrically had a higher fungal burden and showed signs of more severe mucosal inflammatory hyperplasia in the stomach and colon^[94,95]. These results indicate that IL-22 serves as a protective guardian in regulating inflammatory responses and maintaining mucosal barrier integrity in a variety of intestinal infections. However, IL-22 has also been shown to promote intestinal inflammation in parasite infection^[95]. *Toxoplasma gondii*-infected IL-22 knockout mice and mice whose IL-22 was neutralized with an anti-IL-22 monoclonal antibody developed significantly less intestinal pathology and had less weight loss and mortality, despite having similar parasite burdens to infected WT mice. Perhaps the strongly skewed Th1 immune response caused by the *Toxoplasma gondii* infection may explain this difference.

As mentioned above, IL-22 produced by Th17 cells can be regulated by the gut microbiota. Different from the neutrophil induction response of IL-17, IL-22 serves an important role in tissue repair during mucosal immune system response^[96]. Regardless, the relationship between the intestinal microbiota and IL-22-producing cells is extremely close. Most notably, it was recently shown that IL-22-producing innate lymphocytes play a crucial role in preventing systemic inflammation by inhibiting systemic dissemination of commensal bacteria^[83]. Sonnenberg *et al.*^[97] administrated Rag1^{-/-} mice a neutralizing anti-IL-22 monoclonal antibody, and found that the signs of systemic inflammation increased as did levels of lipopolysaccharide (LPS); in addition, bacteria could be cultured from the spleens and livers of these mice. The disseminated commensal bacteria were subsequently identified as *Alcaligenes* sp.

Therefore, together with the protective role against IBD, IL-22 also serves as a mucosal protector and plays a critical role in separating our intestinal tract from our gut flora. Under gut homeostatic conditions, viable bacterial pathogens are sampled by DCs that carry them to the mesenteric lymph nodes and these microbes do not disseminate systemically to secondary lymphoid tissues, indicating that a mesenteric guardian may act in concert with a mucosal firewall to distinguish intestinal bacteria^[98].

IL-22 in tissue protection, regeneration and wound healing

In addition to its antibacterial activity, IL-22 can enhance the survival and proliferation of epithelial cells for

tissue differentiation and healing^[99]. IL-22 induces the expression of antiapoptotic proteins, including Bcl-xL, Bcl-2 and Mcl-1, as well as proteins directly involved in cell cycle and proliferation, such as c-Myc, cyclin D1, Rb2 and CDK4, and anti-inflammatory or protective proteins, such as IL-11 and follistatin^[100-102]. Moreover, IL-22 has been shown to be capable of stimulating a colonic cancer cell line to express a molecule termed deleted in malignant brain tumor 1 (DMBT1), which may play a vital role in the differentiation of epithelial cells^[103]. IL-22 has also been shown to induce RegI α , which serves as a trophic and antiapoptotic factor in the inflamed colon of UC patients. Recent research has determined that, through the activation of STAT3, IL-22 can induce the proliferation and reconstruction of mucosal epithelial cells in the intestinal tract^[104]. This increased healing response can further prevent the penetration of microorganisms into the intestinal epithelial layers.

IL-22 is associated with IBD susceptible genes

An attractive biological feature of IL-22 is its functional association with some major IBD susceptibility genes. Interaction of IL-23 with IL-23R has been implicated in the development of IL-22-producing innate cells, including ILCs, LT α i cells and NK cells^[4,33,34], and in the maintenance of IL-22-producing Th17 cells^[105,106]. Functional polymorphisms of the IL-23R gene have been negatively correlated with development of both CD and UC^[105,106]. IL-22 is located within a UC-risk locus on chromosome 12q15^[107]. IL-22 can be combined with its receptors that are composed of IL-10R2 and IL-22R1. Polymorphisms of *il110r2* are positively associated with both CD and UC^[108].

Binding of IL-22 with its cognate receptor induces rapid activation of STAT3 through JAK1 and TYK2. *Stat3*, *jak1* and *tyk2* are all well-defined susceptibility genes of CD and, to a lesser extent, of UC^[95,96]. STAT3 activation stimulates epithelial cells to produce Muc1. A recent genome-wide association study proposed *muc1* as a potential candidate gene associated with CD^[109]. In addition, genome-wide association analysis of IBD patients has identified gene mutations involved in encoding IL-22 and the IL-10R2 subunit of the IL-22R complex^[110,111].

IL-22 AS A POTENTIAL THERAPUTIC FOR IBD

Due to its crucial roles in regulating barrier immunity and antimicrobiota, IL-22 may have therapeutic potential for IBD. Understanding the various mechanisms of IL-22 in regulating immunity, together with development of immunosuppressive drugs, may open up a new path for the future treatment of IBD.

Treatment with recombinant cytokine or gene therapy delivery of IL-22 may alleviate tissue damage during inflammatory responses. Suppressing the immune system *via* anti-inflammatory treatments, such as TNF α

inhibition, can lead to unwanted dampening of the immune response, impairing the ability of its response to infection. However, IL-22 is an ideal therapeutic candidate because it specifically affects tissue responses and does not have direct effects on the immune response. IL-22 has produced the expected results in an experimental animal model of IBD. Administration of a more specific targeting agent of IL-22 *via* microinjection of an IL-22 DNA vaccine into already inflamed colonic tissues of mice with IBD has been shown to lead to reduce infiltration of inflammatory cells as well as to increase number of goblet cells^[112]. This enhances the production of mucin, thereby buffering the colonic epithelium from commensal bacteria that may otherwise initiate an immune response. Andoh *et al.*^[113] did not find IL-22 expression in the gut mucosa of patients with infectious colitis. It seems that IL-22 plays a protective systemic role in CD^[114] and a protective local role in UC^[115,116]. It should be mentioned at this point that recently, Leppkes *et al.*^[117] demonstrated that the adoptive transfer of IL-22-deficient T cells into RAG1-deficient mice caused severe colitis that was indistinguishable from that caused by transferred WT cells.

Genome-wide linkage analysis of IBD patients has identified gene mutations involved in encoding IL-22 and the subunit complex of IL-10R2 and IL-22R1^[111]. The IL-22R complex is highly expressed within the gastrointestinal tract and in the inflamed colon; IL-22 is expressed by CD4⁺ T cells, likely Th17 cells, and innate lymphocytes, such as NK cells and LT α i-like cells. Using different experimental models of IBD - DSS-induced colitis, which is thought to be mainly driven by innate immune response cells, and CD4⁺CD45RB^{high} T cell-mediated colitis, in which naive T cells devoid of Tregs are transferred into T cell-deficient mice where they proliferate unimpeded leading to colitis - IL-22 has been shown to be protective^[118,119]. Furthermore, Strengell *et al.*^[118] showed that IL-22 can be therapeutic in IBD; gene therapy transfer of the IL-22 gene into the colons of already inflamed mice resulted in amelioration of inflammation. In addition, the authors reported that *in vivo* gene delivery of IL-22 attenuates Th2-mediated colitis and regulates the expression of genes related to mucus layer formation^[120].

Some existing biologic therapies are also able to mediate effects on IL-22 expression in patients. Anti-TNF α antibodies (such as infliximab) and the anti-IL-6 antibody tocilizumab have been used to treat IBD. Th22 cells depend on TNF α for differentiation; therefore, both Th17 and Th22 cells depend on IL-6 for differentiation, and they can indirectly decrease IL-22 expression in patients to treat IBD^[121,122]. Lastly, ustekinumab is able to target both IL-12 and IL-23 and therefore prevent the differentiation of Th1, Th17 and Th22 cells, eliminating several sources of IL-22; this drug is currently being studied in Phase III clinical trials of CD^[123]. The treatments mentioned above suppress inflammation by indirect inhibition of IL-22.

CONCLUSION

IL-22 plays a critical role in the regeneration of damaged epithelial monolayers and stimulates antimicrobial peptide generation. Importantly, the ability of IL-22 to promote intestinal wound healing and proliferation of intestinal epithelial cells in mice and humans has been reproducibly demonstrated by independent groups using different experimental methods, and recent advances in genome-wide association studies have led to results suggesting that the IL-22 pathway is closely related to some major IBD susceptibility genes. These collective findings clearly highlight IL-22 as a promising target for IBD therapy. Therefore, further extensive research on IL-22 is necessary to bring about novel and practical interventions for improving the quality of life of patients with IBD in a safe and effective way. Further understanding of the regulation and function of IL-22 would certainly play a favorable role in the future treatment of IBD.

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Microscopic colitis: Common cause of unexplained nonbloody diarrhea

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Core tip: The gastroenterologist and gastrointestinal pathologist should keep this common condition in mind as a cause of unexplained nonbloody diarrhea with normal mucosal appearance on colonoscopy to avoid misdiagnosis that may affect treatment of patients. Referral of patients with pathogen negative chronic diarrhea to medical centers that have facilities for colonoscopy and biopsy is vital in the developing world.

Abstract

Microscopic colitis (MC) is characterized by chronic, watery, secretory diarrhea, with a normal or near normal gross appearance of the colonic mucosa. Biopsy is diagnostic and usually reveals either lymphocytic colitis or collagenous colitis. The symptoms of collagenous colitis appear most commonly in the sixth decade. Patients report watery, nonbloody diarrhea of a chronic, intermittent or chronic recurrent course. With collagenous colitis, the major microscopic characteristic is a thickened collagen layer beneath the colonic mucosa, and with lymphocytic colitis, an increased number of intraepithelial lymphocytes. Histological workup can confirm a diagnosis of MC and distinguish the two distinct histological forms, namely, collagenous and lymphocytic colitis. Presently, both forms are diagnosed and treated in the same way; thus, the description of the two forms is not of clinical value although this may change in the future. Since microscopic colitis was first described in 1976 and only recently recognized as a common cause of diarrhea, many practicing physicians may not be aware of this entity. In this review, we outline the epidemiology, risk factors associated with MC, its etiopathogenesis, the approach to diagnosis and the management of these individuals.

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INTRODUCTION

Microscopic colitis is regarded as one of the common causes of chronic watery diarrhea. The incidence rate for collagenous colitis is 0.8/100000-6.2/100000. Many cases have been reported in western countries and in Asian countries like India. Lindstrom and Freeman described the term collagenous colitis concurrently in 1976. Microscopic colitis (MC) refers to two medical conditions which cause diarrhea: collagenous colitis and lymphocytic colitis. The following triad of clinicopathological features characterizes both conditions: (1) chronic watery nonbloody diarrhea; (2) normal mucosal appearance on colonoscopy; and (3) characteristic histopathology.

Patients are characteristically, although not exclusive-

ly, middle-aged females. They present with a long history of watery nonbloody diarrhea which may be profuse. There is a strong association between autoimmune diseases, for example arthritis, Sjorgen's syndrome and celiac disease, and microscopic colitis. There are reports of associations with multiple drugs, especially non-steroidal anti-inflammatory drugs. Colonoscopy is normal or near normal. The changes are often patchy, so multiple colonic biopsies must be taken in order to make a correct histological diagnosis^[1-3]. A full colonoscopy is required as an examination limited to the rectum will miss cases of MC.

EPIDEMIOLOGY

The true incidence of MC is not known. The disease has been increasingly diagnosed over the past 20 years but is still uncommon. A recently published population-based study found the incidence of microscopic colitis to increase significantly from 1.1 per 100000 persons in the late 1980s to 19.6 per 100000 persons by the end of 2001. More recent epidemiological studies done in this century confirmed these high incidence numbers, showing that actual incidence and prevalence numbers are higher than initially thought and are still able to show rising incidences, although the rise is far less pronounced than before. Most recent north American studies show incidence rates of 7.1 per 100000 person-years for collagenous colitis and 12.6 per 100000 person-years for lymphocytic colitis^[4].

Morbidity is limited to the consequences of diarrhea, including metabolic abnormalities such as hypokalemia and dehydration, weight loss and fatigue. This is not considered a life-threatening condition; however, profuse watery diarrhea may lead to severe dehydration and electrolyte abnormalities requiring intensive resuscitation.

Lymphocytic colitis affects similar numbers of men and women, while collagenous colitis is up to 20 times more frequent in women than in men^[5].

Both conditions are observed most commonly in people over the age of 40 years, with peak incidence in the sixth and seventh decades of life, and the incidence of both conditions increases with age. Isolated cases have been reported in younger populations, including children^[5-8].

ETIOPATHOGENESIS

The etiology of MC is most likely multifactorial with a mucosal inflammatory response to yet not specified noxious luminal agent occurring in a predisposed host. The noxious luminal agent may be a single one or multiple ones summing up to an individual threshold. Technically, MC is an inflammatory bowel disease (IBD) and the disease shares a number of etiological aspects with the so-called classical inflammatory bowel diseases like Crohn's disease and ulcerative colitis. Among the possible predisposing and/or contributing factors for microscopic colitis, genetic factors and intraluminal noxious factors

are best studied. Although a limited number of familial clusters of microscopic colitis have been reported, there is only minimal evidence of a genetic component within the etiology of MC. All reported so-called family clusters are very small and comprise of a maximum of two reported family members. In contrast, there is evidence of a predisposition of sensitivity to gastrointestinal inflammatory insults in patients with microscopic colitis since up to 12% of patients with MC have a family history of celiac disease or even inflammatory bowel disease^[8]. The meaning of the association between Human Leukocyte antigen (HLA-DQ2, DQ1, DQ3) and microscopic colitis and the high prevalence of a tumor necrosis factor (TNF α) gene polymorphisms in patients with microscopic colitis deserves further attention as it may lead to a discovery of a hereditary component of microscopic colitis of presently unknown penetration^[9]. Furthermore, metalloproteinase-9 gene variations have been reported to be associated with collagenous colitis^[10]. However, the meaning of all the presently reported genetic associations is poorly understood and the respective research is presently not driven by hypotheses, rather than by incidental observations or genetic screening. Very strong evidence exists for an autoimmune basis to the development of both collagenous and lymphocytic colitis. The association of MC with autoimmune-based disorders such as celiac, thyroid disease and rheumatoid arthritis, as well as the female preponderance, supports the notion that both forms of MC have a strong association with autoimmune diseases and may well be an autoimmune disorder themselves. However, to date, no specific autoantibody has been identified as being diagnostic for or being associated with collagenous or lymphocytic colitis^[11]. It is known that MC can be found together with various autoantibodies and phenotypes, like human leukocyte antigen (HLA)-DR3 phenotype, although these associations are not strong enough to be regarded as being diagnostically relevant or useful, nor do we know what these associations mean. A case series of 4 patients is described in which subjects developed classic symptoms of lymphocytic-collagenous colitis with typical mucosal histopathology during treatment with omeprazole/esomeprazole (proton pump inhibitors)^[12]. Symptoms promptly stopped and mucosal biopsies returned to normal with drug withdrawal. Disease quickly recurred in 2 patients who were re-exposed to the drugs, one with biopsy documented recurrent collagenous colitis. Luminal factors of whatever kind seem to play an important role in the pathogenesis of microscopic colitis. Numerous drugs were reported to have a high or at least intermediate probability of causality in microscopic colitis^[13]. Other luminal factors like infectious or even toxic agents are supported by studies that found either onset of MC following a gastrointestinal infection or improvement of symptoms with the initiation of antibiotics in the context of a proven or suspected gastrointestinal infection. *Yesinia* species^[14], *Clostridium difficile*^[15] and *Campylobacter* species^[16] were suggested in published case reports to cause MC; although, interpreting these observations in the context of

current knowledge, it is most likely that these cases are of sporadic nature. In some small retrospective case series, bile acid malabsorption was found in up to 60% of patients with lymphocytic and up to 44% of patients with collagenous colitis, supporting the notion that MC may at least in some patients be caused by bile acid malabsorption. Whether bile acid malabsorption is causative or not remains questionable, as later studies were unable to confirm these observations^[17]. Still, this may direct therapeutic decisions and especially in patients with a cholecystectomy, a bile acid directed treatment should be considered. Basic science is still in its infancy when it comes to studying microscopic colitis and possible causes, drivers, mechanisms or even pathophysiological models. A recent bench side study employing sigmoid tissues from patients with collagenous colitis and lymphocytic colitis was able to identify that sodium transport and epithelial barrier function are disturbed in patients with microscopic colitis^[18]. Unfortunately, it remains unclear whether these reported changes are of causal nature, of transient nature, or a consequence of the underlying microscopic colitis. Even although these descriptive studies at least initiate a scientific discussion on what may be mechanisms underlying or involved in the development and the resolution of microscopic colitis, these small mechanistic studies have to be carefully taken since such studies are highly artificial in the techniques they use and therefore the results are most likely influenced not only by numerous circumstances like laboratory procedures and protocols but also by patient drug use, age and even nutritional status. Thus, such information has to be considered as hypotheses generating information that hopefully guides future prospective studies that help us to understand the mechanisms involved in the pathogenesis, maintenance and resolution of microscopic colitis and symptoms associated with the histological changes.

Using molecular techniques, it was reported that in patients with MC, increased interferon- γ , TNF- α and IL-1 β levels suggest a Th1 cytokine profile being involved in the inflammatory process^[18]. The differences in mucosal lymphocyte subsets seen in patients with collagenous and lymphocytic colitis^[19] is not fully understood presently. This information may help us to understand the inflammatory mechanisms involved and may be useful for future therapeutic approaches. Environmental factors may play a crucial role in the etiology of MC, although other than cigarette smoking, presently no other factors are confirmed. For both collagenous and lymphocytic colitis, cigarette smoking is more prevalent compared to subjects without MC and first reports suggest that lung cancer is associated with MC^[20-22]. The odds ratio (OR) for lymphocytic colitis and smoking (OR = 3.8) is higher than for collagenous colitis and smoking (OR = 2.4), although this difference was calculated on a small cohort of 120 patients with collagenous colitis, 70 patients with lymphocytic colitis, and 128 controls, and thus has to be verified in larger groups of patients^[23]. Interestingly, it was additionally shown that MC occurs roughly 10 years earlier when the respective person is an active

smoker, stressing the relevance of cigarette smoking to the pathophysiology of microscopic colitis. Beyond the strong results from association studies, it would be of great impact to learn whether cessation of smoking would cure MC or at least be beneficial to the patient's symptoms, and a prospective clinical trial answering this seems worthwhile. In addition to the inflammatory component in the pathophysiology of MC, there may be an additional neuronal component to pathophysiology. A recent study identified increased chromogranin A, chromogranin B and secretoneurin levels in feces of patients with collagenous colitis compared to relevant control groups. These observations may point to a neurogenic involvement in MC and, additionally, these stool markers are suggested to be helpful in discriminating MC from irritable bowel syndrome or classical inflammatory bowel disease^[24].

The precise mechanism of diarrhea in these patients is not well understood. Factors that may play a role include bile salt induced injury, active chloride excretion, decrease in net sodium absorption, creation of a diffusion barrier by collagen band and increased local inflammatory mediators such as nitric oxide and prostaglandins. It remains unclear which of these results in the symptoms reported by patients. Two studies have looked at inflammatory cytokines in MC. Patients with MC seem to have a predominantly TH1 type cytokine profile with significant increases in interferon gamma, TNF- α and interleukin 15, as well as an increased inducible nitric oxide synthetase. Others have found increased levels of Transforming growth factor- β in patients with collagenous colitis^[25].

CLINICAL PRESENTATION

Collagenous and lymphocytic colitis present with very similar symptoms and from a clinical perspective, there is no specific symptom or clinical feature that allows discriminating one from the other. Thus, the differentiation between the two entities is made by histology only. The typical clinical presentation involves chronic (either recurrent or intermittent) relapsing watery, nonbloody diarrhea. Symptoms may have been present for several months to 2-3 years before medical attention is sought and a diagnosis is made. Less frequent complaints include abdominal cramping, fecal incontinence and weight loss, although weight loss may be seen in 40% or more of patients with collagenous colitis. Incontinence is probably more a reflection of the advanced age of those individuals who are affected and patients with this problem may do well if treated with antidiarrheal agents^[26,27]. The natural history of MC is variable. Many cases are self-limiting, with symptoms lasting a few weeks or months. Others may be symptomatic for years in a relapsing or continuous pattern. Although a small number of case reports have suggested that MC may lead to development of ulcerative colitis, a small case series of patients with MC showed that none developed ulcerative colitis or Cohn's disease after a follow-up of at least 6 years^[28]. There are case reports on spontaneous and colonoscopy induced



Figure 1 Micrograph of collagenous colitis, a type of microscopic colitis showing sub epithelial band of collagen H and E stain.

colonic perforations in patients with MC^[29-30].

DIAGNOSIS/HISTOPATHOLOGY

The diagnosis of MC is dependent on (1) a convincing clinical history with other etiologies ruled out; (2) normal or near normal endoscopic and/or radiographic findings; and (3) endoscopic biopsies with histopathological findings consistent with MC.

The first step in the diagnostic process is a thorough history with particular attention paid to risk factors and the disease associated with MC. A complete history helps one to rule out other etiologies that may cause a similar clinical picture, such as IBD, celiac disease, diarrhea-predominant irritable bowel syndrome or infectious colitis.

Laboratory and radiographic investigations can be employed to rule out other entities on the differential diagnosis list but they are typically unremarkable.

Endoscopy with biopsy is necessary to arrive at the diagnosis. Colonoscopy generally reveals normal mucosal appearance. However, non-specific changes such as erythema, edema, abnormal vascular markings or even tears associated with perforation have been described. The hallmark of microscopic colitis is an increase in inflammatory cells (*i.e.*, lymphocytes) in colonic biopsies with an otherwise normal appearance and architecture of the colon. Inflammatory cells are increased both in the surface epithelium ("intraepithelial lymphocytes") and in the lamina propria. In lymphocytic colitis, these are the only abnormal features.

In collagenous colitis, the features of lymphocytic colitis are present, with the additional presence of a characteristic thickened sub epithelial collagen band which may be up to 30 μ m thick (Figure 1)^[31].

As the mucosa is not ulcerated or otherwise disrupted, the diarrhea generally does not contain blood or pus^[32]. The diarrhea in collagenous colitis is likely due to inflammatory process and sub epithelial collagen serves as a cofactor in the role of a diffusion barrier and increased levels of immunoreactive prostaglandins E2 in stool water may lead to secretory diarrhea. Some cases may have fibrosis due to increased mucosal secretion of vascular endothelial growth factor^[32,33]. One important question is how many biopsies need to be taken and how

many biopsies are needed to confirm or rule out microscopic colitis. Numerous studies showed that the microscopic lesions can be skipped and therefore random multiple colonic biopsies should be taken^[34].

Treatment

Treatment recommendations for MC are largely based on case reports and uncontrolled studies. Specific agents evaluated include 5-aminosalicylic acid (5-ASA), prednisone, immunomodulators, bismuth, probiotics and *Boswellia* extract. Small randomized controlled trials have shown that agents such as budesonide offer promise as an effective form of symptomatic therapy for both collagenous and lymphocytic colitis. As a first step in managing MC, an in depth medication history should be taken with potentially precipitating medications stopped where possible. Associated conditions such as celiac disease should be appropriately managed. In patients with mild symptoms, dietary restrictions like avoiding caffeine and lactose might be helpful.

Anti-diarrheal therapies

Non-specific anti-diarrheal therapies such as loperamide are commonly used in the management of MC. Retrospective studies have suggested benefit with doses ranging from 2 to 16 mg/d^[34]. Due to the safety of this agent and the possibility of spontaneous remission, loperamide is the first-line therapy for MC.

Aminosalicylates

Uncontrolled retrospective series have suggested symptomatic improvement in up to 50% of patients with MC treated with mesalamine (5-ASA). A recent randomized trial of 64 MC patients compared mesalamine (800 mg *tid*) to mesalamine (800 mg *tid*) and cholestyramine (4 g/d). Treatment resulted in resolution of diarrhea in 84% overall after 2 wk. If treatment was continued over 6 mo, clinical and histological remission was achieved in 85% of those with lymphocytic and 91% of those with collagenous colitis. The number of relapsing patients after 6 mo of treatment was low and symptomatic relapses could be successfully retreated. Overall, the combination of mesalamine with cholestyramine was slightly superior^[35,36].

Budesonide

Budesonide is currently the most promising treatment for collagenous colitis. Three trials involving 94 patients have shown that budesonide therapy (9 mg/d for 6-8 wk) compared to placebo resulted in statistically significant improvements in clinical symptoms and quality of life. A recent Cochrane database meta-analysis reported pooled OR of 12.3 for clinical response with budesonide with a number needed to treat of two. Although effective in the short-term, all trials showed a high rate (61%-80%) of relapse within 2 wk of budesonide cessation. Age < 60 years was a significant risk factor for relapse. Although there are no studies to support a tapering course of budesonide, many clinicians employ this in an effort to

minimize the likelihood of relapse.

One randomized controlled trial of budesonide for the treatment of lymphocytic colitis has been conducted. When compared to the placebo arm, the patients randomized to budesonide (9 mg/d \times 6 wk) had a statistically significantly higher rate of remission (< 3 bowel movements per day) at 3 and 6 wk^[37-41].

Prednisolone

A double-blind, placebo-controlled randomized trial of oral prednisolone 50 mg/d for 2 wk for collagenous colitis was inconclusive because of the low number of patients enrolled^[42]. Studies examining the effect of prednisone in the treatment of lymphocytic colitis have not been performed.

Immunosuppressive therapy

Immunosuppressive therapy with azathioprine or methotrexate has been utilized in patients either refractory to corticosteroid therapy or corticosteroid dependent, but there are no randomized controlled trials to guide therapy with these medications.

Other therapies

Small clinical trials studying bismuth subsalicylate, *Boswellia serrata* extract, probiotics and empirical antibiotic treatment for collagenous and lymphocytic colitis look promising but cannot be suggested outside of such trials. Finally, case reports suggest that pentoxifylline, verapamil and subcutaneous octreotide might be treatment options, but their use cannot be recommended at this time. When medical therapy was unsuccessful and symptoms were very severe, surgical interventions, such as a temporary or permanent loop ileostomy or even a proctocolectomy, have been employed in smaller case series.

CONCLUSION

To conclude, the term microscopic colitis is now used to describe both lymphocytic and collagenous colitis and the condition should be kept in mind in any patient with unexplained watery nonbloody diarrhea with normal endoscopic findings. Biopsy is a must to rule out either form of microscopic colitis. Based on symptom severity and disease duration, a stepwise approach to treatment is suggested.

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"Mucosal healing" in ulcerative colitis: Between clinical evidence and market suggestion

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Core tip: In recent years, the concept that the management of ulcerative colitis patients should aim to heal the mucosa rather than resolve symptoms has been decisively proposed. Herein, we review the current evidence supporting this statement and analyze the possible practical implications in the current management of ulcerative colitis patients.

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Abstract

In recent decades, the prominent role of endoscopy in the management of ulcerative colitis (UC) has been translated into the concept of mucosal healing (MH) as a fundamental therapeutic end-point. This is partially the consequence of growing evidence of a positive prognostic role of MH on the disease course and partially due to market cues indicating a higher rate of MH in patients treated by novel potent biologic agents. The aim of the present review is to clarify the current knowledge of MH in UC, analyzing the definition, the putative prognostic role and the association of MH with the current drugs used to treat UC patients. Because solid data about the management of UC patients based solely on the healing of the mucosa are not yet available, a tailored approach for individual patients that considers the natural history of UC and the presence of prognostic indicators of aggressive disease is desirable. Consequently, unnecessary examinations and treatment would be avoided and restricted to UC patients who require the maximum amount of effort to affect the disease course in the short and long term.

INTRODUCTION

A crucial topic that physicians have long faced in the management of ulcerative colitis (UC) patients is the identification of a reference parameter for the assessment of disease activity. Indeed, UC is a chronic inflammatory disease of the colon, characterized by limitation of the inflammation to the mucosa and the proximal extension of the disease starting from the rectum^[1]. Indeed, the term "UC" comprises a heterogeneous condition with differing involvement of the colon in terms of its extension and the grade of inflammation, which in turn can lead to possible alterations of laboratory parameters and symptom occurrence and severity. The clinical, biochemical and mucosal alterations do not always directly correlate, and questions have been raised about which parameter should be used as the "gold standard" for disease activity assessment.

For the capacity to directly evaluate the colon, which is the target organ of the disease, endoscopy has been indicated as the more accurate tool to assess the activity of the disease, further supported by the possible misleading role of symptoms in the evaluation of UC patients^[2,3]. Unfortunately, colonoscopy is an invasive, costly and time-consuming procedure, and the routine repetition of the examination is not feasible. Different objective surrogate parameters have been described to aid physicians in the correct evaluation of the activity state of patients with inflammatory bowel disease (IBD), including serological (*i.e.*, C-reactive protein) and fecal (*i.e.*, calprotectin, lactoferrin) markers^[4], as well as clinical scores^[5].

During the last decade, the addition to the IBD therapeutic arsenal of anti-TNF- α biologic drugs, which were formerly used in other chronic inflammatory conditions, has launched a “Copernican revolution” in the clinical approach to both Crohn’s disease (CD) patients and UC patients. In fact, together with a rapid and consistent improvement of symptoms and laboratory parameters, such potent anti-inflammatory compounds have resulted in rapid and dramatic improvements of the intestinal mucosal lesions characteristic of IBD, as documented by endoscopic evaluation before and after the induction therapy^[6]. Since this time, the relevance of the endoscopic activity of disease has been definitively stated, and “mucosal healing” (MH) has been proposed with increasing strength as a fundamental therapeutic goal of IBD treatment, claiming its prognostic relevance in the natural history of the disease^[3]. Since the first studies that described the efficacy of Infliximab in CD patients^[7,8], some 15 years have passed, and the therapeutic options for IBD patients have consistently expanded. At present, two biologic anti-TNF agents are currently approved in Europe for utilization in both CD and UC (Infliximab and Adalimumab), and some biologic agents have already shown efficacy in randomized clinical trials and are indicated for market release^[9]. The emphasis on the efficacy of such novel drugs for the amelioration of mucosal inflammation has contributed to making the concept of MH a paramount therapeutic goal, and we are passing from a symptom-targeted to a mucosa-targeted approach in the management of IBD patients^[10]. Several observations have contributed to encourage this shift in IBD management, outlining the relation between mucosal healing and the favorable long-term outcome of the disease in terms of reductions in flares, hospitalizations, the need for surgery and cancer incidence^[11].

Although the MH concept has recently been particularly emphasized in CD, the importance of endoscopic remission in UC has been known for a long time^[12]. In fact, the achievement of MH in UC appears of particular relevance for the localization of the disease (mucosal and limited to the colon), which renders the endoscopic examination relatively easier compared with CD, in which the inflammation is transmural and can potentially involve areas of the intestine not accessible to endoscopic inspection. Considering that more than half of

UC patients present inflammation limited to the left side of the colon^[13], the possibility that the involved areas can be easily scoped to evaluate MH in such patients is particularly tempting.

Nonetheless, specific treat-to-target studies addressing the effective role on the natural progression of the disease of a treatment strategy focused electively on the achievement of MH are still lacking. The relevance of MH to the management of UC, although intriguing and rational, remains to be firmly established. The possibility that the importance of MH would tend to be overrated due to the influence of sponsored trials underlining the association between MH and biologic drugs must be considered. Moreover, data coming from randomized clinical trials (RCTs) are usually not completely applicable to the “real-life” IBD population. In fact, a recent retrospective analysis of consecutive mild-moderate IBD patients at a United States tertiary referral center found that only 31.1% of patients would fulfill the inclusion criteria of the major RCTs of biologic agents and that the outcomes of patients fulfilling the criteria are significantly more favorable compared with those not meeting the criteria^[14].

Besides scientific and commercial suggestions, a careful revision of the actual evidence in support of MH is essential. The risk of a blind and excessively enthusiastic adherence to the MH suggestion is concrete, and physicians need to be aware of the over-prescription of unnecessary endoscopic examinations and/or the over-treatment of patients. In an era of resource optimization, this would risk minimizing the same advantages that the MH strategy is claiming, *i.e.*, the reduction of disease costs by reducing complications and hospitalizations. Extensive systematic reviews of MH are already available in the literature (*i.e.*, Neurath *et al.*^[11]), and such a review is beyond the aim of the present work. Here, we intend to perform a synthetic and careful revision of the state-of-the art research on MH. To this end, we critically reviewed the definition of MH, the quality of the actual evidence of its prognostic relevance, and the capacity of the therapies currently used for UC to achieve MH, with the final goal of clarifying the potential correct application of the concepts of MH to the current practical management of patients affected by UC.

MH: DEFINITION

Although a standardized definition of MH has not been established, a practical currently accepted definition is “the complete resolution of the visible alterations or lesions, irrespective of their severity and/or type at baseline colonoscopy”^[11]. Nonetheless, at present, an easy to use, validated and clinically relevant endoscopic score for UC activity evaluation is lacking, reflecting the complexity in measuring disease activity in UC^[15]. In fact, although a great number of scoring systems have been developed (Baron score, Mayo score, Sutherland, Powell-Tuck and Rachmilewitz indices, among others)^[16-24], none of them have been prospectively validated. The main problems

regarding the majority of the indices include the overlap of mucosal features (such as vascularity, granularity, erythema, friability, bleeding, and ulceration), leading to inter-observer variation in endoscopic evaluation, and the lack of clear and standardized thresholds for endoscopic remission or improvement. The Mayo Clinic endoscopy subscore has been the most commonly used in recent clinical trials, defining MH as a score of ≤ 1 (normal mucosa or loss of vascular pattern, but no mucosal friability), when the endoscopy subscore was 2 or 3 at baseline. The problem of a standardized definition of MH is not theoretical but implies concrete and practical consequences. In fact, in recent clinical trials, heterogeneous definitions may have contributed to the higher rate of patients with MH when compared with that of patients achieving clinical remission^[25], although alternative explanations are possible (*e.g.*, the simultaneous presence of irritable bowel syndrome, dysmotility). Moreover, a recent RCT testing the use of mesalamine in UC patients showed consistently different results after a revision of the endoscopic examination findings by a blinded central reader^[26].

In further support of the aforementioned difficult evaluation of UC endoscopic activity, two novel scores have been very recently developed and prospectively validated, the Ulcerative Colitis Endoscopic Index of Severity and the Ulcerative Colitis Colonoscopic Index of Severity^[27,28]. Data about the applicability of such new scores in clinical trials and in clinical practice are awaited and will hopefully aid the move toward a standardized definition of MH.

Recently, data indicating a prognostically relevant role for histologic activity in the mucosa of UC patients, in addition to the macroscopic activity, have opened the door to the concept of “histological MH”, with the complete absence of clinical, laboratory, endoscopic and histological features of active inflammation^[29]. Indeed, the term “mucosal healing” was initially proposed only for the disappearance of the inflammatory infiltrate in the histological examination^[30]. At present, although some scoring systems for histologic activity have been described, none have been properly validated or commonly used, and therefore, the definition of histological MH remains without consensus.

MH: EVIDENCE FOR PROGNOSTIC RELEVANCE

The increasing relevance of the MH achievement in UC has been demonstrated by a growing body of data showing the different courses of the disease in patients with and without MH, with a reduction of complications such as flares as well as reductions in hospitalization, colectomy and cancer incidence in patients with MH.

As early as 1966, Wright *et al.*^[31] reported a higher relapse rate in patients who did not achieve MH after oral and rectal steroids when compared with patients who did achieve MH (40% *vs* 18%). In the ACT1 and ACT2

trials, patients treated with infliximab who exhibited MH at week 8 showed a higher rate of clinical remission at week 30 than patients without MH (48.3% *vs* 9.5%)^[32]. Yamamoto *et al.*^[33] reported that UC patients who achieved clinical remission and MH after leukocytapheresis had a higher rate of sustained clinical response when compared with patients with only a clinical response (88% *vs* 41%). Ardizzone *et al.*^[34] showed that the lack of mucosal healing at 3 mo after the first corticosteroid treatment was the only factor associated with negative outcomes at 5 years (use of immunosuppressants, hospitalization and colectomy).

An observational study of the IBSEN cohort showed that in 513 UC patients, the colectomy rate was lower in patients with MH [defined by a simple endoscopic score of 0-1 (0, normal; 1, light erythema or granularity)] at a 5-year follow-up (2% *vs* 8%, $P < 0.05$)^[35]. Similar results were shown by Soldberg *et al.*^[36], who reported a decrease in the colectomy rate in UC patients with MH at 1 year after diagnosis, regardless of the therapy used to achieve it, and in a post-hoc analysis of the ACT1/ACT2 trials conducted by Colombel *et al.*^[37], in which a Mayo Clinic endoscopy subscore of 0-1 in Infliximab-treated patients was related to a lower probability of colectomy than a score of 2-3 through a follow-up period of 54 wk. Interestingly, in the latter article by Colombel *et al.*^[37], MH in the placebo group did not show the same positive prognostic value as it did in the Infliximab-treated group, questioning the prognostic value of MH “*per se*” and suggesting that the drugs used to achieve the MH may play a specific role in the long-term outcome.

The increased risk of colorectal cancer incidence in UC patients is still a matter of debate^[38]. Nonetheless, the inflammatory burden appears to be an important determinant, and consequently, MH is likely to reduce the risk. An Italian cohort study indicated a lower CRC risk at 17 years of follow-up in azathioprine (AZA)-treated UC patients with MH^[39].

Recently, appealing data have indicated a possible prognostic role for histologic remission in terms of reductions in flares, surgery/hospitalization and CRC incidence, suggesting histologic remission as the ultimate therapeutic goal in UC management^[29]. In fact, Bitton *et al.*^[40] have reported basal plasmacytosis at rectal biopsy as an independent predictor of early relapse in UC patients, and Bessisow *et al.*^[41] have described a higher rate of flares in patients with macroscopically healed mucosa but histologic activity when compared with patients with both the macro- and microscopic absence of disease. Nonetheless, correlations with macroscopic and microscopic activity are not always straightforward^[42], and routine biopsies are not suggested by the current guidelines. At present, more evidence is needed before considering histological MH as a possible goal of treatment in UC patients.

MH: CURRENT THERAPIES

Biologic agents

As mentioned, the MH concept has been clearly defined

Table 1 Randomized clinical trial of biologic agent in ulcerative colitis and the relative mucosal healing rates

Ref.	Patients (n)	Treatment protocol duration	Evaluation time from baseline	MH rate
Rutgeerts <i>et al</i> ^[32]	728	IFX 5 or 10 mg/kg every 8 wk	Week 8	60.7% IFX
		Placebo		32.3% placebo
		30 wk (ACT2)	Week 30	50.6% IFX
		54 wk (ACT1)	Week 54	27.4% placebo 46.0% IFX 18.2% placebo
Panaccione <i>et al</i> ^[50]	231	AZA 2.5 mg/kg	Week 16	37% AZA
		IFX 5 mg/kg		55% IFX
		IFX 5 mg/kg + AZA 2.5 mg/kg		63% AZA + IFX
Sandborn <i>et al</i> ^[25]	494	ADA 160/80 and then 40 mg eow	Week 8	41.1% ADA
		Placebo		31.7% placebo
		52 wk	Week 52	25.0% ADA 15.4% placebo
Reinisch <i>et al</i> ^[43]	390	ADA 160/80 mg or 80/40 mg at weeks 0 and 2 followed by 40 mg at weeks 4 and 6	Week 8	46.9% ADA (160/80)
		Placebo		37.7% ADA (80/40)
		52 wk	Week 52	41.5% placebo 54% ADA
Feagan <i>et al</i> ^[44]	225	VED 300 mg at week 0 and 2 and then every 4 or 8 wk	Week 6	40.7% VED
		Placebo		24.8% placebo
		52 wk	Week 52	56% VED (every 4 wk) 51.6% VED (every 8 wk) 19.8% placebo
Sandborn <i>et al</i> ^[45,46]	774	GOL 400/200 or 200/100 mg at weeks 0 and 2 followed by 50 mg or 100 mg every 4 wk	Week 6	45.1% GOL (400/200)
		Placebo		42.3% GOL (200/100)
		54 wk	Week 54	28.7% placebo 42.4% GOL100 every 4 wk 41.7% GOL 50 every 4 wk 26.6% placebo

MH: Mucosal healing; IFX: Infliximab; ADA: Adalimumab; AZA: Azathioprine; VED: Vedolizumab; GOL: Golimumab.

only in the biologic era, and the trials of biologic drugs present a better evaluation of this aspect than previous studies. In particular, the MH definition has been standardized by the utilization of the Mayo endoscopic subscore, which identifies as MH as a score of 0 or 1. However, MH has always been considered as a secondary end-point in clinical trials, and studies still present heterogeneity in terms of inclusion criteria (and, therefore, baseline endoscopic severity), design and follow-up. Nonetheless, the MH rates in the short (induction) and long term (maintenance) are consistent and significantly superior to those of placebo in all studies (Table 1), which is even more remarkable considering the baseline severity of the UC patients included, although, in most of the studies, MH was only observed in a minority of the patients^[25,32,43-46]. Moreover, as mentioned, patients in RCTs are superselected, and the results may be not directly applicable to the “real-life” IBD population.

Azathioprine

From the first early report by Jewell *et al*^[47] of increased MH after 4 wk in UC patients treated with corticosteroids plus AZA *vs* corticosteroids plus placebo (92% *vs* 71%, *P* = ns), few studies with a limited number of patients have addressed MH rates in AZA-treated UC patients. In all of the reported studies, MH was a secondary end-

point, and the MH definition, base-line endoscopic activity, timing of the endoscopic evaluation and concomitant therapies differed; therefore, conclusive results are hard to extrapolate.

With the aforementioned limitations, Paoluzi *et al*^[48] reported 57% and 45% rates of MH in UC patients treated with AZA at 6 mo (*n* = 42 patients) and 4 years (*n* = 22 patients), respectively, and a similar 6-mo rate was reported by Ardizzone *et al*^[49] [19/36 patients treated with AZA (53%) *vs* 7/36 of patients treated by 5ASA (19%)]. Recently, a study by Panaccione *et al*^[50] (available only in abstract form) reported a 36% MH rate in patients treated with AZA in monotherapy and a 63% MH rate in patients treated with AZA plus Infliximab at 4 mo, with nearly 80 patients per group, indicating that combination therapy may increase the rate of MH.

Corticosteroids

Unlike CD, in which corticosteroids are traditionally considered ineffective for the achievement of MH^[51], corticosteroids may induce MH and a clinical response in UC. The first evidence supporting a favorable role of corticosteroids in inducing MH dates back to 1954, when True-love reported a double-blind placebo-controlled randomized multicenter trial of 120 UC patients and demonstrated higher rates of MH in the oral cortisone (100 mg/d) group

than in the placebo group (30% *vs* 10%) within 6 wk^[16].

In the last six decades, a great number of studies have reported positive effects of corticosteroid therapy on the improvement/resolution of mucosal alterations in UC, irrespective of the route of administration (oral or rectal) and the type of corticosteroids (traditional systemic steroids or agents with low systemic availability)^[52-59]. Generally, a certain discrepancy between the clinical and endoscopic responses was present in the majority of the studies evaluating MH in UC after corticosteroid treatment. A meta-analysis by Marshall *et al*^[55] examining the role of rectal corticosteroid preparations showed similar clinical (approximately 45% of cases) and endoscopic (approximately 33% of cases) remission rates for conventional corticosteroids (hydrocortisone, prednisolone, methylprednisolone and betamethasone) and topically active corticosteroids (beclomethasone, budesonide and prednisolone metasulphobenzoate). Recently, Ardizzone *et al*^[34], in a study of 157 consecutive newly diagnosed UC patients, explored the potential prognostic significance of a 3-mo clinical and endoscopic response after the first course of corticosteroid treatment. After 3 months, 60 patients (38.2%) had a complete clinical and endoscopic response, 39 (24.8%) had a clinical but not an endoscopic response, and 58 (36.9%) had no response. Interestingly, failure to achieve endoscopic remission at the end of the first course of steroids was related to a more aggressive disease behavior.

Data obtained from the use of topical steroids present a reduced variability between clinical and endoscopic responses. Indeed, in a recent meta-analysis exploring the efficacy of rectal beclomethasone dipropionate, the clinical and endoscopic rates of improvement or remission were similar (65.3%) and concordant, although in the four trials considered for the meta-analysis, a clear definition and evaluation of mucosal healing were lacking^[60].

Several problems arise in the attempt to analyze and compare the results of the above-mentioned studies. Diversity in the timing of endoscopy and in the use of endoscopic indices (*e.g.*, Sigmoidoscopic score, Rachmilewitz index, Baron score) along with the lack of a univocal MH definition, possible inter-observer variations or heterogeneity of the included patient cohorts may have generally contributed to consistent variability in the MH rates in steroid trials.

Aminosalicylates

Mesalamine was approved by the Food and Drug Administration in late 1987, and since this time, it has become the cornerstone therapy for mild-moderate UC^[61]. Mesalamine can be administered orally and/or topically, and it is present on the market in different formulations specific to both methods of administration. Many studies show the ability of mesalamine to induce MH. A recent meta-analysis of 49 studies has concluded that MH is achieved in approximately 37% and 50% of patients treated with oral and topical mesalamine, respectively^[62]. Nonetheless, the results from single studies are dramatically different,

ranging from approximately 0% to 77% for oral mesalamine^[23,63] and from approximately 10% to 93% for topical formulations^[54,64]. This variability may be attributed to the different definitions of MH, but this is unlikely to be the only reason. While MH rates do not appear to be related to the release mechanisms of oral mesalamine^[62], in accordance with previous studies reporting similar effectiveness between different formulations^[61,65,66], studies continue to present great heterogeneity in terms of total dose in grams, disease extension, months of follow up and endoscopic score at baseline. Notably, the MH rates in placebo groups are reported to be high, up to 46% in a study of oral placebo *vs* oral mesalamine at 8 wk^[63] and 26%-37% in a study of topical placebo *vs* topical mesalamine after 6 wk^[67]. Moreover, in studies with therapeutic regimens of adequate dose and duration, the MH rate appears to be higher^[68-70], and the lack of achievement of MH in patients with clinical remission has been indicated as a possible negative prognostic factor for relapse occurrence^[71].

CONCLUSION

After the emergence of novel biologic therapies for UC, the old concept of the relevance of the endoscopic activity of disease has been translated into the new concept of MH as the therapeutic goal to achieve. Although this idea has been supported by a growing body of scientific evidence indicating the favorable prognostic value of a healed mucosa in the natural history of UC, it is also suggested commercially, as a high rate of MH is claimed when utilizing the new biologic agents. Indeed, endoscopic evaluation appears to be the “gold standard” for the evaluation of disease activity in UC patients, and healing of the mucosa is likely to be an important factor for the control of the disease in the short and long term. However, specific studies showing the superiority of a management based solely on MH over the “traditional” approach are lacking. To date, most of the evidence supporting the prognostic relevance of MH comes from studies in which MH is not considered as the primary endpoint as well as from retrospective investigations. In the present study, we provocatively addressed the issue of the relevance of MH for UC patients management. A careful review of the current evidence regarding MH in UC shows that, due to the high heterogeneity of the available studies (particularly for those from the pre-biologic era), crucial points are still far from being conclusively determined, including the MH definition, the expected rate of MH with the current medication, and whether a systematic assessment of MH and an optimization of therapy based on MH alone would improve long-term disease outcome. Moreover, the prognostic value of MH “*per se*” needs to be investigated to clarify whether the current drugs may be safely reduced or interrupted after MH achievement. The latter issue may also present consistent economic implications regarding the elevated cost of long-term maintenance therapy with biologic drugs. However, in most cases, MH appears to be achiev-

able only in a minority of UC patients and most likely with the utilization of potent and potentially dangerous therapeutic regimens. In the near future, the development of novel drugs and an increase in our knowledge of the complexity of IBD are desirable, as they may increase the efficacy of our therapeutic approach to the disease.

Notably, going back to the natural history of the disease, more than one-half of UC patients have a benign disease course, while up to one-third are likely to experience frequent flares and potentially dangerous complications. In fact, the large population study by Solberg *et al.*^[36] (IBSEN cohort), which evaluated the first 10 years of the disease course in a population of 519 patients with UC, highlighted an overall good prognosis. Their study showed that at 10 years, more than half of patients were in remission or had mild disease, while 37% and 6%, respectively, reported chronic intermittent and chronic continuous symptoms. In a large Danish cohort study, approximately one-third of patients had no flares within 10 years after the first attack of UC. Moreover, the cumulative probability of having a course without relapses after 10 years in patients in remission is 40%-60%^[72]. However, the colectomy rate is estimated to vary from 8.7% to 30% in different populations^[72-74], and after the first relapse, the cumulative rates of a second course of systemic steroids are 13%, 41% and 48% at 1, 5 and 10 years, respectively^[36].

In times of resource optimization, the ideal disease management would imply an aggressive treatment and endoscopic follow-up for the achievement of MH in patients with an unfavorable disease course. Accordingly, together with a better definition of the MH concept and its specific role in the management of UC patients, further research for the characterization of clinical and/or genetic features predictive of an aggressive behavior of the disease is urgently needed. Similarly, the identification and the implementation of clinical and laboratory parameters strongly correlated with the endoscopic activity, such as clinical scores, to better follow-up these patients, appear to be of relevance^[75]. Consequently, it is advisable that the aforementioned shift from a symptoms-based to a mucosa-based approach in the management of UC patients would not result in a trend to over-scope and/or over-treat patients for the achievement of MH. Indeed, because more solid evidence will be available regarding the role of MH, a rational approach to UC patients should reserve close monitoring and more potent therapies for “high-risk” patients, overcoming the dualism between symptom- and mucosa-targeted approaches and focusing increasingly on a “patient-based” approach.

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Implication of miRNAs for inflammatory bowel disease treatment: Systematic review

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Abstract

Inflammatory bowel disease (IBD) is believed to develop *via* a complex interaction between genetic, environmental factors and the mucosal immune system. Crohn's disease and ulcerative colitis are two major clinical forms of IBD. MicroRNAs (miRNAs) are a class of small, endogenous, noncoding RNA molecules, and evolutionary conserved in animals and plants. It controls protein production at the post-transcriptional level by targeting mRNAs for translational repression or degradation. MiRNAs are important in many biological processes, such as signal transduction, cellular proliferation, differentiation and apoptosis. Considerable attention has been paid on the key role of miRNAs in autoimmune and inflammatory disease, especially IBD. Recent studies have identified altered miRNA profiles in ulcerative colitis, Crohn's disease and inflammatory bowel disease-associated colorectal cancer. In addition, emerging data have implicated that special miRNAs which suppress functional targets play a critical role in regulating key pathogenic mechanism in IBD. MiRNAs were found involving in regulation of nuclear transcription factor kappa B pathway (*e.g.*, miR-146a, miR-146b, miR-122, miR-132, miR-126), intestinal epithelial barrier function

(*e.g.*, miR-21, miR-150, miR-200b) and the autophagic activity (*e.g.*, miR-30c, miR-130a, miR-106b, miR-93, miR-196). This review aims at discussing recent advances in our understanding of miRNAs in IBD pathogenesis, their role as disease biomarkers, and perspective for future investigation and clinical application.

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Key words: Crohn's disease; Inflammatory bowel disease; MicroRNA; Treatment; Ulcerative colitis; Biomarker

Core tip: MicroRNAs (miRNAs) are a class of small, noncoding RNA molecules that post-transcriptionally regulate gene and protein expression. Recent studies have identified altered miRNA profiles in inflammatory bowel disease (IBD). Special miRNAs which suppress functional targets have been found to play a critical role in regulating key pathogenic mechanism in IBD. In this review, we discuss the possibility to use miRNAs as biomarkers and therapeutic target in IBD.

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INTRODUCTION

Inflammatory bowel disease (IBD) refers to chronic remittent or progressive inflammatory conditions that may affect the entire gastrointestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) are two major clinical forms of IBD^[1]. The incidence and prevalence of IBD is continuously increasing over the past decades in different regions around the world^[2]. Although the precise pathogenesis of IBD remains obscure, several reports have

indicated that dysfunction of the mucosal immune system which develops *via* a complex interaction between genetic factors, the host immune system and environmental factors plays an important role in its etiology^[3]. The chronic inflammation of IBD is associated with marked molecular changes in gene and protein expression^[4]. So small molecules targeted at the pathways involving in these processes may be potential for IBD diagnosis and treatment.

MicroRNAs (miRNAs) are considered as promising candidate. They are a class of single-stranded non-coding RNA molecules on an average 22 nucleotides long^[5], and are highly conserved throughout evolution^[6] and discovered in all eukaryotic cells except fungi^[7]. MiRNAs regulate gene expression both at a transcriptional and translational level^[8], and mediate post-transcriptional gene silencing by directly binding to the 3' untranslated region (UTR) of target mRNA. Depending on the level of sequence complementarity between miRNA and target site, mRNA transcripts targeted by miRNAs are either silenced if the base-pair match is imperfect or degraded if there is an identical base-pair match^[9]. The mRNAs inhibited by miRNAs move to cytoplasm and accumulate in cytosolic processing bodies until they are eventually degraded^[10]. Each miRNA can target hundreds of genes, and a particular gene is usually the target of multiple miRNAs, adding complexity to the regulation of gene transcriptional network^[11]. It has been reported that miRNAs play an important role in many biological processes, such as signal transduction, cellular proliferation, differentiation, apoptosis and immune response^[12,13]. Recently, miRNAs have been recognized as critical elements in the regulation of the innate and adaptive immune responses, and changes in miRNAs expression are related to many autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, psoriasis and IBD^[14-17].

In this review, we summarize the current understanding of the connection between miRNAs and IBD. We mainly focus on special dysregulated miRNAs in CD and UC, which lead to inappropriate expression of targeted mRNA and may contribute to IBD pathogenesis, diagnosis and treatment. Table 1 summarizes the altered miRNAs involved in IBD and their mRNA targets.

MIRNAS REGULATE NUCLEAR TRANSCRIPTION FACTOR KAPPA B PATHWAY

The nuclear transcription factor kappaB (NF- κ B) was identified as one of the important regulators in the immune system and inflammatory diseases^[18]. NF- κ B is markedly induced in IBD patients and strongly influences the course of mucosal inflammation through its ability to promote the expression of various pro-inflammatory genes^[19]. Nucleotide-binding oligomerization domain 2 (NOD2) was found to be the first IBD susceptibility gene^[20], which is mainly expressed in Paneth cells, monocytes, macrophages, dendritic cells and some types of

intestinal epithelia cell^[21]. NOD2 can be activated by muramyl dipeptide (MDP), a component of bacterial cell wall, which induces the activation of NF- κ B^[22].

MiR-146a was reported to regulate gut inflammation *via* NOD2-sonic hedgehog (SHH) signaling^[23]. SHH signaling is an important pathway that maintains gut homeostasis and directs gut development. The expressions of NOD2-induced iNOS and NO were increased in MDP-treated macrophages, which further induced the level of miR-146. Promoter luciferase analysis with miR-146a promoters revealed that NF- κ B was a critical transcription factor that regulate NOD2 mediated expression of miR-146a. NOD-2 induced miR-146a target NUMB, a negative regulator of SHH signaling, alleviating the suppression of SHH signaling and subsequently increasing the pro-inflammatory cytokines expression.

Feng *et al*^[24] proved that up-regulation of miR-126 may contribute to pathogenesis of UC by targeting I κ B α . They found miR-126 was significantly increased in active UC tissues compared to healthy controls. I κ B α , an inhibitor of NF- κ B pathway and the target of miR-126, was markedly decreased in active UC tissues. The expression of miR-126 and I κ B α were inversely correlated in patients with active UC. MiR-126 could inhibit the level of I κ B α in HT29 cells. They further demonstrated that miR-126 may activate NF- κ B signaling pathway by targeting I κ B α and contribute to the development of UC. Another study showed that the anti-inflammatory activities of the red wine polyphenolics were, at least in part, mediated by the induction of miR-126^[25]. CAMs, such as intracellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), are expressed on the surface of fibroblasts^[26]. It has been demonstrated that the expression of ICAM-1 was increased in CD patients^[27] and inhibition of CAMs could suppress various forms of experimental inflammatory and immune responses in colon fibroblast cells^[28]. VCAM-1 has been confirmed as one of the targets of miR-126 before^[29]. Angel-Morales *et al*^[25] found the polyphenolic red wine extract (WE) exerted an anti-inflammatory effect in LPS-stimulated human colon-derived CCD-18Co myofibroblast cells through inactivating NF- κ B and down-regulating a wide range of downstream pro-inflammatory genes including tumor necrosis factor (TNF)- α , interleukin-6 (IL-6) and CAMs. Furthermore, they found the up-regulation of miR-126 was induced by WE in CCD-18Co cells and protected human colon cells from inflammation through targeting VCAM-1.

MiR-122 was found dysregulated in association with CD progression^[30]. Chen *et al*^[31] identified NOD2 as a target of miR-122. Overexpression of miR-122 in LPS-stimulated HT-29 cells inhibited LPS-induced apoptosis and down-regulated LPS-induced NOD2 expression. Pretreatment with miR-122 in LPS-stimulated HT-29 cells decreased the pro-inflammatory cytokines and increased the anti-inflammatory cytokines by targeting NOD2-induced NF- κ B signaling pathway. Taken together, miR-122 might decrease intestinal epithelial cell injury in Crohn's disease by targeting NOD2. Besides regulating the activation of NF- κ B pathway, Ye *et al*^[32] demonstrated

Table 1 List a core set of altered microRNAs involved in inflammatory bowel disease and their mRNA targets

miRNA	Target mRNA	Net effect	Ref.
Increased expression			
miR-146a	NUMB	SHH signaling upregulation	[23]
miR-146b	Siah2	NFκB signaling upregulation	[40]
miR-126	IκBα	NFκB signaling upregulation	[24]
	Vascular cell adhesion molecule-1	Suppresses proinflammatory cytokines	[25]
miR-122	Nucleotide-binding oligomerization domain 2	Decreases intestinal epithelial cell injury	[31]
	Occluding	Intestinal permeability upregulation	[32]
miR-132	AChE	Decreases circulation AChE activity	[37]
miR-21	RhoB	Impairment of tight junctions	[52,53]
miR-150	c-Myb	Promotes apoptosis	[54]
miR-141	CXCL12β	Regulates leukocyte migration	[62]
miR-106b	ATG16L1	deregulation of autophagy	[67,68]
miR-196	IRGM	deregulation of autophagy	[70]
miR-30c	ATG5	inhibition of autophagic activity	[69]
miR-130a	ATG16L1	inhibition of autophagic activity	[69]
Decreased expression			
miR-10a	IL-12/IL-23p40	Regulates intestinal homeostasis	[43]
miR-124	STAT3	Promotes inflammation	[48]
miR-200b	ZEB1, SMAD2	Regulates epithelial-mesenchymal transition	[59,60]
miR-192,miR-495, miR-512,miR-671	NOD2	NFκB signaling upregulation	[34]

NF-κB: Nuclear transcription factor kappa B; NOD2: Nucleotide-binding oligomerization domain 2; AChE: Acetylcholinesterase; IRGM: Immunity-related GTPase family; IL: Interleukin; ZEB1: Zinc finger E-box binding homeobox 1; SMAD2: SMAD family member 2.

the involvement of miR-122 in the regulation of intestinal epithelial tight junction (TJ) permeability. Deficient intestinal epithelial TJ barrier, characterized by the increase of intestinal permeability, has been demonstrated to contribute to the development of IBD as an important pathogenic factor^[33]. MiR-122 was significantly increased in TNF-α-stimulated Caco-2 cells and induced the increase in Caco-2 TJ permeability by targeting occluding. The up-regulation of intestinal permeability by miR-122 was proved *in vivo* as well^[32]. Based on the two studies, miR-122 plays a complex and controversial role in the development of IBD.

Chuang *et al.*^[34] showed that NOD2 expression is regulated by miRNAs in HCT116 cells. They found that MDP could induce the expression of NOD2 and activate the NF-κB signaling pathway in HCT116 cells. MiRNAs targeted NOD2, such as miR-192, miR-495, miR-512 and miR-671, were significantly decreased in MDP-stimulated HCT116 cells, which had an inversely correlation with the expression of NOD2. Overexpression of these NOD2-associated miRNAs in MDP-stimulated HCT116 cells inhibited the activity of NF-κB and the downstream pro-inflammatory cytokines, IL-8 and CXCL3.

MiR-132 was a potential regulator of acetylcholinesterase (AChE) activity in inflammatory condition and was shown to target AChE to reduce its activity *in vitro* and in mouse models^[35]. Acetylcholine (ACh) activates its receptor on macrophage through which it interrupts the nuclear translocation of NFκB and suppresses the production of pro-inflammatory cytokines^[36]. Maharshak *et al.*^[37] found miR-132 had an anti-inflammatory effect on the development of IBD. MiR-132 level was significantly upregulated in biopsies from patients with IBD compared with controls. In accordance with this, circulation AChE ac-

tivity was significantly lower in patients with IBD suffering from moderate-severe disease. These data implicated a possible regulation of AChE activity by increased miR-132 levels, which eventually ameliorated inflammation in patients with IBD.

Although NFκB was originally thought to be an almost exclusively pro-inflammatory player in the setting of IBD, its role in epithelial cells was confirmed more controversial. Several studies using knockout mice with defective NF-κB activation have demonstrated an anti-inflammatory function of NFκB in colonic epithelial cells^[38,39]. Nata *et al.*^[40] showed that miR-146b, another member of miR-146 family, can alleviate intestinal injury in mouse colitis *via* the activation of NF-κB and the improvement of epithelial barrier function. MiR-146b was found significantly up-regulated in IL-10 deficient mice. The whole sequence of miR-146 was intraperitoneally administered to the dextran sodium sulfate (DSS)-induced colitis mouse. Overexpression of miR-146b in DSS-induced colitis mouse activated NFκB, relieved intestinal inflammation, improved epithelial barrier function, and increased the survival rate. Furthermore, the protective effect of miR-146b on mouse with DSS-induced colitis was negated by inhibition of the NFκB pathway. Siah2, which was the target of miR-146b, promoted ubiquitination of TRAF proteins upstream of NFκB. It suggested that miR-146b up-regulated NFκB *via* suppressing siah2, which finally improved intestinal inflammation.

MIRNAS REGULATE IL-23/IL-23R PATHWAY

IL-23, a heterodimeric cytokine comprising IL-12p40

and IL-23p19, is produced by activated macrophages, monocytes, DCs and endothelial cells. IL-23 receptor is composed of IL-12Rβ1 (shared with the IL-12 receptor) and the specific IL-23R subunit. IL-23 acts on the IL-23 receptor and promotes expansion and maintenance of Th17 cells, which secrete the pro-inflammatory cytokine IL-17 and have been implicated in the pathogenesis of many chronic inflammatory disorders, including IBD^[41,42]. MiRNA was considered as a new mechanism in regulating the IL-23/TH17 pathway and subsequent downstream IL-17 production in IBD.

Xue *et al.*^[43] found much lower expression of miR-10a in intestinal epithelial cells and dendritic cells of specific pathogen-free mice compared to germ-free mice. IL-12/IL-23p40 was identified as a target of miR-10a. They further demonstrated that microbiota negatively regulated host miR-10a expression by targeting IL-12/IL-23p40, which may contribute to the maintenance of intestinal homeostasis.

IL-23R gene variants have been identified as risk factors for IBD^[44]. The rs10889677 variant in the 3'UTR region of IL-23R gene which led to a loss of binding capacity for let-7e and let-7f displayed increased expression of IL-23R^[45]. It means this mutation sustained IL-23 signaling and contributed to chronicity of IBD. Furthermore, Li *et al.*^[46] showed let-7f down-regulated the expression of IL-23R and its downstream cytokine IL-17 by targeting IL-23R.

MIRNAS REGULATE IL-6/STAT3 PATHWAY

Previous studies have shown the importance of the IL-6/STAT3 signaling pathway in IBD. Inhibition of IL-6/STAT3 cascades results in the suppression of acquired immune mediated colitis^[47]. Koukos *et al.*^[48] found miR-124 were significantly decreased in colon tissues from children with UC and mice with experimental colitis, and the levels of STAT3 and its regulated genes were up-regulated simultaneously. They demonstrated reduced levels of miR-124 in colon tissues of pediatric patients with active UC might increase expression and activity of STAT3 by direct binding to its 3'UTR, which could promote inflammation and the pathogenesis of UC in children.

MIRNAS REGULATE INTESTINAL EPITHELIAL BARRIER FUNCTION

The intestinal mucosal barrier, of which the intestinal epithelial cells are the most integral part, maintains a delicate balance between absorbing essential nutrients while preventing the entry and responding to harmful subjects^[49]. Dysfunction of intestinal epithelial barrier has been extensively reported in IBD^[49,50].

Disruptions of important elements of the intestinal barrier in IBD lead to permeability defects^[51]. There were two studies showed that miR-21 played a pro-

inflammatory role in IBD by impairing intestinal barrier function. Yang *et al.*^[52] found levels of miR-21 were up-regulated in both the mucosal and serum of patients with UC. RhoB, which was the target of miR-21 and involved in modulating intestinal epithelial permeability, was found significantly decreased in the patients with UC. They demonstrated that overexpression of miR-21 in patients with UC and Caco-2 cells impaired intestinal tight junction integrity and morphology through targeting RhoB. Similarly, Shi *et al.*^[53] reported that miR-21 was overexpressed in IBD patients, IL-10 KO mice and DSS-treated mice. MiR-21 knockout (KO) mice was less susceptible to experimental colitis and had more ameliorative inflammatory responses than wild type (WT) mice. Moreover, the increase of Intestinal permeability and epithelial cells apoptosis induced by DSS were attenuated in miR-21 KO mice.

Bian *et al.*^[54] found miR-150 was significantly elevated, whereas c-Myb, a target of miR-150, was strongly decreased in colon tissue of UC patients and DSS-treated mice. Overexpression of miR-150 in HT29 cells enhanced cell apoptosis through targeting c-Myb, which damaged intestinal epithelial barrier.

Epithelial-to-mesenchymal-transition (EMT) is characterized by losing epithelial cell markers such as E-cadherin and gaining mesenchymal proteins including vimentin, which enhances invasiveness, migratory capacity and production of cell-extracellular matrix components^[55,56]. Recent studies demonstrated that EMT contributed to the loss of intestinal epithelial cells (IECs) and subsequent increased intestinal paracellular permeability and decreased intestinal epithelial barrier function^[57,58]. Chen *et al.*^[59] found miR-200b significantly decreased in inflamed mucosa in IBD patients, which was positively correlated to the expression of E-cadherin and negatively correlated to the level of TGF-β1 and vimentin. Overexpression of miR-200b in TGF-β1-stimulated IEC-6 cells increased E-cadherin and decreased vimentin through targeting zinc finger E-box binding homeobox 1 and SMAD2 respectively, which prevented TGF-β1-induced EMT. Intestinal fibrosis is a common serious complication of CD. In another study, they demonstrated that miR-200b could partially protect intestinal epithelial cells from fibrogenesis by suppressing EMT *in vitro*^[60]. In summary, miR-200b played a potential role in maintaining intact of intestinal epithelium through inhibiting EMT and improving pathophysiology and clinical outcomes of IBD.

MIRNAS REGULATE COLONIC EPITHELIAL CELL-DERIVED CHEMOKINE EXPRESSION

The expression of intestinal epithelial-derived CXC and CC chemokines is increased in IBD^[61]. Huang *et al.*^[62] found up-regulated level of miR-141 was inversely correlated with CXCL12β in the epithelial cells of the inflamed colon tissues from CD patients and mice with experimental colitis. They further demonstrated that miR-141 directly regulated CXCL12β expression and leukocyte migration

mediated by CXCL12 β . Additionally, overexpression or knockdown of miR-141 in the colon of mice with experimental colitis regulated leukocyte infiltration and alleviated or aggravated intestinal inflammation, respectively. Wu *et al.*^[65] found miR-192 was decreased in active UC and demonstrated an inverse relationship between miR-192 and MIP-2 (CXCL2).

MIRNAS REGULATE AUTOPHAGY

Autophagy, which is involved in recycling cellular organelles for the survival of cell, is one mechanism for maintaining cellular hemostasis. Autophagy in the intestinal epithelium is considered to behave as a defensive strategy for clearance of intracellular microorganisms, and the impairment of autophagy results in intestinal epithelial dysfunction and contributes to IBD pathogenesis^[64]. *ATG16L1* and *IRGM*, two genes associated with autophagy, have been identified as CD susceptibility genes by genome-wide association studies^[65,66]. Some studies showed that miRNA-mediated change in the expression of autophagy gene may result in autophagy dysfunction and involve in the pathogenesis of IBD.

Lu *et al.*^[67] found that silencing of *Dicer1* enhanced autophagy-related gene (*ATG*) protein levels and autophagosome formation in cells, indicating that miRNAs may be implicated in the regulation of autophagy. MiR-106b and miR-93, which target *ATG16L1*, both reduced levels of autophagy in epithelial cells. MiR-106b could also inhibit autophagy-dependent clearance of CD-associated adherent-invasive *Escherichia coli* (AIEC) in epithelial cells. Inflamed mucosae from subjects with active CD exhibited more overexpressed miR-106b and lower expression of *ATG16L1* when compared with controls. These results suggested that CD patients with miR-106b and miR-93 mediated down-regulation of *ATG16L1* expression might manifest an altered antibacterial activity of CD-associated intracellular bacteria in epithelial cells and subsequently affected the outcome of intestinal inflammation. Similarly, Zhai *et al.*^[68] showed miR-106b targeted *ATG16L1* and modulated autophagic activity in HCT116 cells. Their results further indicated that miR-106a and miR-106b could influence the expression of other autophagy-related genes and had a widespread modulating effect on the autophagy pathway.

Nguyen *et al.*^[69] proved miR-30c and miR-130a directly regulated the expression of *ATG5* and *ATG16L1*, respectively, by targeting their 3'UTRs. They found miR-30c and miR-130a expression were increased and *ATG5* and *ATG16L1* mRNA expression were decreased in non-inflamed or inflamed ileal CD biopsy specimens compared with normal controls. Similarly, the expression of miR-30c and miR-130a were inversely correlated with *ATG5* and *ATG16L1* in intestinal epithelial T84 cells infected with the AIEC. NF- κ B pathway was activated in AIEC infected T84 cells, which induced the up-regulation of miR-30c and miR-130a and consequently inhibited the expression of *ATG5* and *ATG16L1*. The inhibition of autophagic activity by miR-30c and miR-

130a increased AIEC persistence within T84 cells and enhanced pro-inflammatory cytokines production. Furthermore, they demonstrated inhibition of miR-30c and miR-130a *in vivo* suppressed AIEC-induced down-regulation of *ATG5* and *ATG16L1* expression and increased autophagic activity, leading to more efficient intracellular bacteria clearance and decreased inflammation.

Brest *et al.*^[70] demonstrated that the association of IRGM with CD arised from a miRNA-based alteration in IRGM regulation which led to the deregulation of autophagic efficacy. They found a synonymous variant in IRGM (c.313C > T), which was classified as non-causative before, altered a binding site for miR-196. MiR-196, was overexpressed in the inflammatory intestinal epithelia of patients with CD and down-regulated the IRGM protective variant (c.313C) but not the risk-associated allele (c.313T). Subsequent deregulation of IRGM-dependent autophagy compromised control of intracellular replication of CD-associated AIEC and affected the outcome of intestinal inflammation.

MIRNAS ASSOCIATION WITH IBD CARCINOGENESIS

The development of IBD-associated dysplasia and colorectal cancer represents a major complication in patients with IBD^[71,72]. The important role miRNAs played in carcinogenesis is becoming clearer because miRNAs have been referred to the regulation of cancer-related cellular processes, including differentiation, apoptosis, cell cycle progression and immune function^[10]. Growing evidence implicated that miRNAs are also involved in IBD-associated carcinogenesis.

Ludwig *et al.*^[73] showed up-regulated level of miR-21 in IBD-associated dysplastic lesions compared to active IBD patients, which was inversely correlated with the expression of *PDCD4*, a newly characterized tumor suppressor gene. Olaru *et al.*^[74,75] found expressions of miR-224 and miR-31 increased successively at each stage of IBD progression from non-inflamed to inflamed non-neoplastic, dysplastic and finally cancerous mucosae. MiR-224 and miR-31 levels could accurately discriminate normal or chronically inflamed IBD tissues from cancers. They further identified miR-224 regulated cell cycle through targeting p21 and miR-31 regulated tumor angiogenesis by targeting factor inhibiting hypoxia inducible factor 1, both of which subsequently participated in IBD-associated carcinogenesis.

FUTURE PERSPECTIVE IN IBD DIAGNOSTIC AND TREATMENT

Investigations described above showed that special miRNAs suppressing functional targets played a pro-inflammatory or anti-inflammatory role in regulating the pathogenic mechanism of IBD, including activation of NF- κ B, increased intestinal epithelial permeability, abnormal autophagic activity and so on. It means inflam-

matory response, intestinal epithelial barrier and other mechanisms involved in IBD can be regulated by targeting miRNAs, indicating the potential of miRNAs as therapeutic targets for IBD. Besides studying the function of IBD-associated miRNAs *in vitro*, some researchers had administrated miRNAs into mice with experimental colitis by different methods to investigate their functional and therapeutic effect *in vivo*. Inhibition of miR-30c and miR-130a in mice by ileal loop assay suppressed AIEC-induced down-regulation of ATG5 and ATG16L1 expression and decreased intestinal inflammation^[69]. Over-expression of miR-146b in DSS-induced colitis mouse *via* intraperitoneal injection relieved intestinal inflammation and increased the survival rate of mouse^[40]. MiR-141 intracolonic administration in the colon of TNBS-induced and IL-10 KO mice regulated leukocyte infiltration and alleviated intestinal inflammation^[62]. These data showed the effective ways to administrate miRNAs into human and the possibilities for the future clinical applications of miRNA-based therapeutic approaches in IBD.

There have been several studies that identified altered miRNA profiles in both serum and inflamed tissue in patients with UC and CD compared with controls, which have been reviewed by Coskun *et al.*^[76]. Circulating miRNAs in serum exist in membrane vesicles, such as exosomes^[77], or form a complex with lipid protein carriers, such as high-density lipoproteins (HDL)^[78]. So these circulating miRNAs are protected from blood RNases and relatively stable compared with mRNA and protein, which make themselves serving as ideal noninvasive blood biomarkers in patients with IBD. In addition, the aberrant expression of miRNAs in inflamed tissues of patients with UC could also help in IBD diagnosis.

CONCLUSION

MiRNAs are a class of potential gene regulators of critical importance in the pathogenesis of IBD. It has been demonstrated that miRNAs have the possibility to be used as biomarkers and therapeutic target in IBD. Although our knowledge about the miRNAs regulation of IBD has considerably advanced over the last several years, multiple areas warrant future investigation. Most studies have focused on one miRNA which targets a single mRNA. One area worth future investigation is a key miRNA targeting multiple mRNAs or several miRNAs combination targeting a key mRNA. The other area worth future investigation focuses on the roles of miRNAs in human studies. Most of our understanding of the functions of miRNAs associated with IBD is based on cell cultures and murine models. Further investigating the roles of miRNAs in the human context will improve our knowledge of miRNAs in the pathogenesis and diagnosis of IBD and pave the way for miRNA-based therapies.

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Laparoscopic surgery in the management of Crohn's disease

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INTRODUCTION

Crohn's disease is an autoimmune disorder that causes chronic transmural inflammation of the gastrointestinal tract and makes up one of the two main components of inflammatory bowel disease^[1]. The terminal ileum and proximal colon are the most frequently affected and initial diagnosis is made early, between the ages of 20-30^[2]. Despite the advances in medical therapies with increasingly new immunomodulator use, the rate of refractory disease requiring surgery has not changed over the years^[3]. Surgery is still common and up to 80% of patients with Crohn's disease will require an operation during their lifetime, with 15%-20% requiring an operation within the first year after diagnosis^[4-6]. Of those patients that undergo surgery, studies have shown that approximately 40%-50% will likely need additional surgical intervention within 10-15 years^[7,8]. The likelihood of a second surgery within one's lifetime is high, with several studies having identified the median age of first surgical resection to be in a patient's third decade^[6,9].

Initially, laparoscopic surgery was not attempted for Crohn's disease due to the intraoperative characteristics that made a laparoscopic approach challenging. These findings often included extensive inflammation, enteric fistulae, thickened mesentery, and skip lesions throughout the bowel^[10]. This belief has changed over time and laparoscopy has become increasingly accepted in patients with Crohn's disease as the use of laparoscopy in a majority of gastrointestinal procedures has become standard^[11]. Crohn's patients are typically young and benefit from a laparoscopic procedure that reduces scar and adhesion formation. In addition, given their high risk of surgical recurrence, Crohn's patients benefit from

Abstract

Crohn's disease is a chronic inflammatory bowel disease with surgery still frequently necessary in its treatment. Since the 1990's, laparoscopic surgery has become increasingly common for primary resections in patients with Crohn's disease and has now become the standard of care. Studies have shown no difference in recurrence rates when compared to open surgery and benefits include shorter hospital stay, lower rates of wound infection and decreased time to bowel function. This review highlights studies comparing the laparoscopic approach to the open approach in specific situations, including cases of complicated Crohn's disease.

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Key words: Crohn's; Laparoscopy; Surgery; Colon; Ileum

Core tip: Laparoscopy is now increasingly used in cases of Crohn's disease. Recurrence rates are similar to that of open surgery and studies have shown benefits of decreased hospital stay as well as earlier bowel function. This review highlights several studies that looked at patients who underwent ileocolic and colon resections as well as more complicated cases of Crohn's.

surgical approaches that maximize abdominal wall integrity^[2,10,12,13].

This article review will evaluate surgical resections as well as common surgical scenarios commonly seen with Crohn's disease and compare the laparoscopic and open approaches. A search was conducted in the PubMed, Cochrane, MEDLINE, and Scopus libraries with the following individual and combined key words: Crohn's disease, laparoscopy, surgery, cost, colon, ileocolic, fistula, recurrent, small bowel, outcome, minimally invasive surgery, inflammatory bowel disease, randomized, metaanalysis. References cited in the articles retrieved were also searched in order to identify other potential sources of information. The results were limited to human studies available in English.

LAPAROSCOPIC ILEOCOLIC RESECTIONS

One of the first randomized trials comparing laparoscopic resections to open resections for refractory ileocolic disease was published in 2001 by Milsom *et al*^[14]. Sixty patients were randomized to undergo either laparoscopic or open procedures. The authors reported improved morbidity rates and hospital length of stay rates in the laparoscopic group, although the anastomotic leak rate was similar between the two groups. Length of surgery favored the open group. Long term follow up showed no difference between the groups in terms of disease recurrence rates.

A similar long term prospective study undertaken from 1999-2003 in the Netherlands showed similar results of no difference in overall disease recurrence between the laparoscopic and the open groups. Additionally, there were fewer incidences of small bowel obstruction and incisional hernias in the laparoscopic group. Overall, patient quality of life and cosmesis scores favored the laparoscopic group^[15].

One of the weaknesses of these randomized prospective studies is that the overall number of patients treated was small. However, metaanalysis studies with a larger number of subjects show that these findings for laparoscopic surgeries are consistent. In a large meta-analysis by Tilney, data from over 15 different studies looking specifically at laparoscopic ileocolic resections was compiled. The analysis included 783 patients, 338 (43.2%) of which had undergone laparoscopic resection. The overall conversion rate to open surgery was 6.8%. As seen in earlier studies, overall surgery duration was longer in the laparoscopic group with a difference of 29.6 min. Perioperative complications and anastomotic leak rates were similar between the two groups. Benefits of laparoscopy were significantly shorter time till bowel function was regained and a shorter hospital stay by 2.7 d^[16].

These findings are also supported by other smaller prospective and retrospective studies comparing open vs laparoscopic ileocolic resections in patients with Crohn's disease. There were no differences in morbidity and mortality.

Furthermore lengths of time till return of bowel function and hospital stay were consistently shorter in the laparoscopic groups^[17-20].

More recently, data reviewed from the National Surgical Quality Improvement Program from 2005-2009 compiled perioperative results from over 1900 ileocolic resections for Crohn's disease, 34% of which were performed laparoscopically. On multivariate analysis, the laparoscopic group was associated with an overall decrease in major and minor perioperative complications as well as a significant decrease in overall hospital stay by 1.08 d^[21].

Long term studies following open and laparoscopic ileocolic resection patients showed no difference in recurrence rates^[22,23]. In one study, the average time to recurrence was 60 mo in the laparoscopic group and 62 mo in the open group. Another study reported the average five year recurrence rates to be 29.1% in laparoscopic patients and 27.7% in open patients. Median times to recurrence were 48 and 56 mo, respectively. These times were not significant with a *P*-value of 0.9104. Of note, the laparoscopic group was found to have lower bowel obstruction rates over that time period^[18].

LAPAROSCOPIC COLON RESECTIONS

Much of the literature focuses on laparoscopic surgery at the ileocolic region. Because Crohn's disease can affect any part of the gastrointestinal tract, other anatomical locations can pose different challenges. Acute colitis rates in Crohn's disease patients ranges from 5% to 10%^[24]. Given the larger size of the colon and potentially broader thicker diseased mesentery of the colon, laparoscopic surgery for Crohn's colitis was slower to become accepted.

One of the earliest studies comparing minimally invasive surgery in Crohn's colitis to open surgery was in 2007. This study case matched 27 patients based on various patient factors including comorbidities and types of surgery, looking at patients only with disease in the colon. The authors found that although overall surgery was longer in the laparoscopic group, complications and estimated blood loss were the same in both groups. Length of hospital stay was significantly shorter in the laparoscopic group when 30 d readmissions were included^[25].

Another study retrospectively looked at 92 patients with Crohn's disease that underwent minimally invasive colon resections. Forty-three cases (47%) were total colectomies, 17 (18%) were subtotal colectomies, 32 (35%) were segmental resections. There were 15 conversions to open resections, but conversions were not associated with longer hospital stay or increased postoperative complications. Five patients required reoperation, three for obstruction and two for anastomotic leak. The only prognostic factor for a complicated hospital course was evidence of perianal disease and 30-d mortality was zero^[26].

One of the largest studies looking at laparoscopic colon resections in patients with Crohn's disease prospectively compared 55 laparoscopic resections to 70 open resections. The conversion rate to open resection of 10.9 was similar to that for ileocolic resections. Of note, 34.5% of patients who underwent laparoscopic surgery had had prior abdominal surgery as compared to 65.7% of the open group. This is one of the weaknesses of this study as surgeon preference dictated which procedure was performed. Although there was likely selection bias, the laparoscopic group was associated with similar benefits that were identified in the randomized studies for ileocolic resections. These benefits included less intraoperative blood loss, shorter hospital stays and quicker return of bowel function after surgery in the laparoscopic group^[27].

LAPAROSCOPIC SURGERY FOR FISTULIZING DISEASE

Enteric fistulas are challenging complications in Crohn's patients as this finding often implies the presence of a large inflammatory mass, a history of prior surgeries, or use of steroids—all of which can make the surgery technically difficult. Surgery for enteric fistulas requires resection of the involved segment and primary anastomosis in the elective setting. Fistulas involving other organs are treated with bowel resection of the involved segment and primary repair of the other involved organ^[10,28]. Some studies have cited intraoperative discovery of an intraabdominal abscess or fistula as an independent risk factor for conversion from a laparoscopic procedure^[29]. In addition, a recent consensus conference was unable to recommend a laparoscopic approach for cases of complex Crohn's disease^[30].

A laparoscopic approach in these patients with complicated Crohn's can be treacherous but as surgeons have become more skilled with laparoscopy, more studies have shown its feasibility. One retrospective review looked at 72 patients who underwent laparoscopic surgery for enteric fistulas. This study included enterocolic, ileo-ileal, enterocutaneous, ileovesical, colovesical, colocutaneous, and colovaginal fistulas. Prior abdominal surgery was present in 39.7% of the patients. Approximately 30% of the patients had multiple fistulas and 12.3% of those underwent multiple resections. The rate of conversion to open resection was low at 4.1% and overall morbidity was 11%^[28].

In a more recent case-matched study 11 patients presenting with 13 fistulas were matched to 22 controls with non-fistulizing disease according to age, sex, nutritional state, steroid use, and type of laparoscopic resection^[31]. Although the sample size was small, the authors were unable to show any difference in operative time, conversion rates, or morbidity rates between the two groups.

A larger prospective comparative study compared laparoscopic ileocolonic resections in patients with complex Crohn's disease (abscess and/or fistula) to pa-

tients without complex Crohn's disease. There was no significant difference in postoperative complications but overall operative time, conversion rates, and frequency of temporary stoma creation were all significantly increased in the complex Crohn's group^[32]. These findings are suggestive of a more challenging operation, although the lack of increased morbidity demonstrates that a laparoscopic approach is still feasible. This is an area that needs to be continued to be studied.

LAPAROSCOPIC SURGERY FOR RECURRENT DISEASE

As indications for laparoscopic surgery in patients with Crohn's disease expands, its use in patients with recurrent disease seems natural. Several studies have compared laparoscopic resection to open resection for recurrent disease with no significant difference in surgical outcomes^[33,34]. The reported conversion rates within these studies ranged from 6.7%-42% with the most common reasons for conversion being adhesions, intraoperative discovery of fistula/abscess, or need for associated bowel resection. In general, the conversion rate for recurrent Crohn's disease was similar to numbers seen in surgeries for initial disease. Only in one study was the conversion rate higher in the recurrent disease group and risk factors for conversion were age greater than 40, repeat resection for recurrent disease, and operative findings of an abscess^[35].

Laparoscopic surgery is also possible in patients whose primary operation was a midline laparotomy. A recent study compared laparoscopic *vs* open surgery for patients with recurrent Crohn's disease where their primary surgery was a bowel resection through a midline laparotomy. The study was a retrospective case matched study comparing 26 patients who underwent laparoscopic resection to 26 patients that underwent open resection. Both groups had comparable demographics in terms of comorbidities and prior number of abdominal surgeries. Of note, the recovery benefits of shorter hospital stay and earlier return of bowel function that are seen in all other studies were not maintained in the laparoscopic group. However, there was a significant decrease in wound complication rates when compared to the open group^[36].

ROLE OF SINGLE INCISION SURGERY IN CROHN'S DISEASE

As the role of laparoscopy has increased in patients with Crohn's disease, other advances such as single incision surgeries have also been studied in these patients. Of the few studies published, there is a significant amount of heterogeneity in terms of the technical aspects of the procedures and long term data is not available. Initial results though, show that the single incision approach is feasible without a large increase in complications and with the benefit of decreased postoperative analgesia.

Other studies have shown that complication profile is similar to laparoscopic surgery with the only advantage appearing to be the decreased number of trocar sites while all other factors were equivalent^[8,37-39].

COST EFFECTIVENESS OF LAPAROSCOPIC SURGERY IN CROHN'S DISEASE

The overall cost of care for Crohn's disease continues to increase, with some estimates placing the annual cost in the United States anywhere from \$10-15.9 billion and \$2.1-16.7 billion in Europe^[40-42]. These estimates are expected to increase as newer biologic drugs are increasingly available and used in management^[43]. Of these costs, hospitalizations accounted for 53%-66% of the total in the United States with an average of \$37459 per hospitalization^[41].

Laparoscopy has the potential to decrease these costs per hospitalization as studies have shown that, when compared to open surgeries, laparoscopic surgeries reduce length of hospital stays and concomitant complications. A recent study comparing laparoscopic to open cases found the difference in hospital charges were significantly different, on average \$27575 *vs* \$38713 respectively^[44]. These savings are consistent with those seen in colorectal cancer resections when comparing laparoscopic to open surgeries^[45]. These savings can be potentially further reduced with the increasing adoption of single port surgery as well^[46].

CONCLUSION

Current literature lacks a large number of randomized trials, but the consistent outcomes seen in the numerous retrospective studies and the small number of randomized studies shows that minimally invasive surgical approaches for Crohn's disease patients are both feasible and safe. It is important to remember that patient selection and surgeon experience are important factors for successful laparoscopic surgery. Complicated Crohn's cases with recurrent disease and enteric fistulas require knowledge of advanced laparoscopic techniques. The primary benefits of laparoscopic surgery over open surgery are quicker return to bowel function, decreased wound infection rates and shorter hospital stays. With no difference in recurrence rates seen, laparoscopy is emerging as the standard approach for patients with Crohn's disease for initial surgery, and even in select cases of patients with recurrent and complicated Crohn's disease.

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***Escherichia coli* in chronic inflammatory bowel diseases: An update on adherent invasive *Escherichia coli* pathogenicity**

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Abstract

Escherichia coli (*E. coli*), and particularly the adherent invasive *E. coli* (AIEC) pathotype, has been increasingly implicated in the ethiopathogenesis of Crohn's disease (CD). *E. coli* strains with similar pathogenic features to AIEC have been associated with other intestinal disorders such as ulcerative colitis, colorectal cancer, and coeliac disease, but AIEC prevalence in these diseases remains largely unexplored. Since AIEC was described one decade ago, substantial progress has been made in deciphering its mechanisms of pathogenicity. However, the molecular bases that characterize the phenotypic properties of this pathotype are still not well resolved. A review of studies focused on *E. coli* populations in inflammatory bowel disease (IBD) is presented here and we discuss about the putative role of this species on each IBD subtype. Given the relevance of AIEC in CD pathogenesis, we present the latest research findings concerning AIEC host-microbe interactions and pathogenicity. We also review the existing data regarding the prevalence and abundance of AIEC in CD and its association with other intestinal diseases from humans and animals, in order to discuss the AIEC disease- and host-specificity. Finally, we highlight the fact that dietary

components frequently found in industrialized countries may enhance AIEC colonization in the gut, which merits further investigation and the implementation of preventative measures.

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Key words: Adherent invasive *Escherichia coli*; Inflammatory bowel disease; Crohn's disease; Pathogenesis; Epidemiology

Core tip: In this review we critically revise the findings on *Escherichia coli* (*E. coli*) populations associated with Crohn's disease and ulcerative colitis. Then we focus on adherent invasive *E. coli* (AIEC), especially in its mechanisms of pathogenicity and epidemiology. We discuss about AIEC disease- and host-specificity and we underline the importance of discovering specific molecular tools to detect AIEC for further epidemiologic studies. Finally we point out to a putative role of diet on AIEC gut colonization.

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ESCHERICHIA COLI IN INFLAMMATORY BOWEL DISEASE

The intestinal microbiota has been implicated in the pathogenesis of Crohn's disease (CD) and ulcerative colitis (UC), the main idiopathic inflammatory bowel diseases (IBDs)^[1]. CD patients demonstrate an altered

intestinal microbial community, and the dysbioses present in colonic CD and ileal CD are different^[2]. In contrast, a specific dysbiosis in UC is starting to be defined, although differences between studies have hampered attempts to reach a clear consensus to date^[2-5]. A number of culture-based and molecular-based studies support the theory that *Escherichia coli* (*E. coli*) is a microbiological factor implicated in CD, but some controversy exists regarding its role in UC^[2,6-17]. In this section, we examine data on *E. coli* populations in CD and UC related to abundance, association with disease activity, translocation of the gut mucosa, and pathogenic features of the strains to highlight the evidence that supports or refutes putative implications for this bacterium in each IBD subtype.

Abundance in the intestinal mucosa and correlation with disease activity

Several independent studies based on quantitative Polymerase Chain Reaction (PCR) have indicated that *E. coli* is augmented in CD patients in comparison with controls^[2,6,11,13]. However, differences are especially significant for CD patients with ileal disease, and no clear association with colonic or ileocolonic CD has been demonstrated. On average, in our cohort, *E. coli* 16S rRNA gene copies accounted for 14% and 33% of total bacteria 16S rRNA gene copies in healthy subjects and ileal CD patients, respectively ($P < 0.001$)^[13]. Of note, a higher abundance of *E. coli* was observed in active CD patients than in patients in remission^[6,11,18]. Accordingly, a previous study using Fluorescent In Situ Hybridization (FISH) demonstrated increased *E. coli* numbers in the epithelium and within the lamina propria in active CD patients compared to inactive CD patients^[14]. In addition, we determined that higher numbers of this species correlated with a reduced amount of time before relapse^[11]. These findings are in agreement with previous data reporting that the higher numbers of *E. coli* isolated from the neoterminal ileum of CD patients are associated with early recurrence of the disease^[7], and that high levels of antibodies against the *E. coli* outer membrane protein C (OmpC) correlate with disease progression, longer duration, and greater need for surgery among CD patients^[19-21].

There is substantial controversy regarding the abundance of *E. coli* in the colonic mucosa of UC patients (Table 1). Several works have consistently reported no increase with respect to healthy subjects^[2,6,7,11-13], arguing against a putative role for *E. coli* in UC, while others have reported increased *E. coli* abundance in UC patients^[8,10,14,16,18,22,23]. As in the majority of these studies both CD and UC patients were analyzed, these controversial observations can not be explained by differences in methodology between studies. We postulate that they can be attributable to differences in the disease severity of the patients included in the studies, as increased numbers of *E. coli* have been associated with activity status in UC patients. Using FISH, epithelium-associated *E. coli*

were found to be more abundant in active UC compared to inactive UC or controls^[14], and quantitative PCR indicated that increased numbers of *E. coli* were present in active UC patients compared to inactive UC patients^[22] as well as in inflamed *vs* non-inflamed UC tissue^[23].

Altogether, substantial evidence supports an overgrowth of *E. coli* in ileal CD patients, while there is still no convincing data that exists for other IBD subtypes. Further studies aimed at comparing the abundance of *E. coli* in IBD patients categorized by disease subtype and assessing any correlation with activity status of the disease would shed light on the role of this bacterium in each IBD subtype and its putative application as a diagnostic and/or prognostic tool.

E. coli localization in the intestinal mucosa

E. coli has been found in the mucus layer, close to the intestinal epithelial cells and in ulcers of both CD and UC patients^[24,25]. Translocation of the intestinal mucosa has been primarily observed in CD^[6] and higher amounts of intracellular *E. coli* were detected in inflamed compared to non-inflamed mucosa^[6,26]. With FISH and immunohistochemistry, *E. coli* has been detected scattered within the lamina propria, either in the extracellular space or inside macrophages, as well as in the subserosal layer, the perivascular areas of the submucosa, the muscle layer, and in germinal centers of lymph follicles of CD patients^[8,14,27]. A recent study using high throughput sequencing indicated a greater proportion of *E. coli* reads in the lymph nodes of ileal CD patients than other CD patients^[28]. Interestingly, *E. coli* DNA was also more frequently found in the granulomas of CD patients (80%) than in non-CD control patients (10%) in a study that used Laser Capture Microdissection and PCR^[29]. In contrast, *E. coli* has not been frequently found to translocate the mucosa of UC patients^[8,24,25], although some controversy exists as some authors have detected *E. coli* in the lamina propria of UC patients^[14,27].

The majority of the aforementioned studies are based on techniques that do not distinguish viable bacteria from dead bacteria. Further studies should study the viability of translocated *E. coli*, particularly in lymph nodes and granulomas, as these locations would be more relevant to establish a link between this bacterium and CD pathogenesis. These studies should also focus on UC patients to clarify the existing controversial data. A lack of *E. coli* translocation in UC would suggest that *E. coli* does not play a primary role in UC pathogenesis or that it plays a different role than in CD.

Pathogenic features of the strains

E. coli strains isolated from IBD patients are clonally diverse^[6,13,17] and belong to distinct serotypes^[6,13,30] and to different sequence types^[6,31-33]. Although a close genetic relationship was detected in a study of IBD pediatric patients^[34], the hypothesis that there is a particular clone associated with IBD has largely been ruled out.

In turn, *E. coli* strains isolated from IBD patients

Table 1 Controversy about *Escherichia coli* imbalances in ulcerative colitis

Ref.	Method	Samples	Comments
Increased <i>E. coli</i> abundance in CD but not UC			
Martin <i>et al</i> ^[12]	Culture	Biopsies	Specially hemagglutinin-positive strains
Martinez-Medina <i>et al</i> ^[13]	qPCR	Biopsies	Specially in ileal CD
Lopez-Siles <i>et al</i> ^[11]	qPCR	Biopsies	Specially in active CD
Darfeuille-Michaud <i>et al</i> ^[7]	culture	Biopsies	Specially in ileal lesions
Baumgart <i>et al</i> ^[6]	qPCR	Biopsies	Specially in ileal CD
Willing <i>et al</i> ^[2]	qPCR	Biopsies	Specially in ileal CD
Increased <i>E. coli</i> abundance in CD and UC			
Mylonaki <i>et al</i> ^[14]	FISH	Biopsies	Specially in active UC patients
Kotlowski <i>et al</i> ^[10]	culture	Biopsies	
Rehman <i>et al</i> ^[16]	cloning	Biopsies	
Fujita <i>et al</i> ^[8]	qPCR	Biopsies	
Schwartz <i>et al</i> ^[18]	qPCR	Feces	Specially in active CD patients
Sha <i>et al</i> ^[22]	qPCR	Feces	Specially in active UC and CD patients
Pilarczyk-Zurek <i>et al</i> ^[23]	qPCR	Biopsies	Specially in inflamed UC tissue

¹Increased *E. coli* abundance in CD with respect to controls but UC patients were not included in the study; ²Increased *E. coli* abundance in UC with respect to controls but CD patients were not included in the study. CD: Crohn's disease; UC: Ulcerative colitis; *E. coli*: *Escherichia coli*.

primarily belong to the B2 and D phylogroups in conjunction with extraintestinal pathogenic *E. coli* (ExPEC). Some works demonstrate major colonization by B2+D phylogroups in IBD patients in comparison with healthy controls^[10,31], but in other studies, a similar distribution of phylogroups exist between IBD and healthy subjects^[13,29,30,33-36]. Differences between studies could be based on the types of samples analyzed, as it has been reported in healthy individuals that transient *E. coli* (more likely to be found in feces) are principally A and B1, whereas resident *E. coli* (more likely to be found in the mucosa) mainly belong to the B2 and D phylogroups^[35]. Therefore, studies based on mucosal samples tend to indicate enrichment of B2 and D strains, even in healthy controls. Another factor that could influence the distribution of phylogroups in IBD is the disease severity of patients analyzed, as an increased proportion of B2 and D isolates has been found in active IBD patients^[32], which was significantly associated with the inflammation state of IBD tissues^[30]. This denotes a shift in *E. coli* populations to isolates that are better adapted to the inflamed tissue in IBD and/or that are involved in the inflammation itself. Of note, no differences in phylogroup distribution between CD and UC have ever been reported.

E. coli isolated from IBD patients carry different sets of virulence genes that are characteristic of ExPEC strains, whereas intestinal pathogenic *E. coli* are rare or absent^[6,10,13,30,32,34,36-39]. These virulence factors are also frequent in *E. coli* from healthy subjects and are considered "colonization factors" necessary for successful establishment in the intestinal mucosa^[40]. Virulence gene profiles are inexorably linked with the phylogenetic origins of the strains. Based on the distribution of phylogenetic groups, virulence-associated genes characteristic of ExPEC were more frequently found in IBD patients than in healthy subjects in those studies where B2+D predominated in IBD^[10,31], whereas no differences were

found in other works^[13,36,37,41,42]. A shift in the phylogroup distribution would then lead to an increased proportion of *E. coli* equipped with colonization factors that would facilitate establishment and persistence in IBD patients. However, it is not clear whether this shift occurs specifically in IBD patients or is a general trend taking place in industrialized countries^[43]. Although no particular genetic traits distinguish *E. coli* from the intestinal mucosa of CD or UC, some virulence factors have been found to be differentially distributed between these IBDs. For example, a diarrhea-associated hemolytic *E. coli* strain called cell-detaching *E. coli* (CDEC), which commonly harbors hemolysin, cytotoxic necrotizing factor 1, pilus P and S-fimbria genes, was found in 24% of UC *E. coli* and only in 4.7% of CD *E. coli*^[44]. The gene *usp* encoding for the uropathogenic-specific protein was also more frequently found in UC *E. coli* than in CD *E. coli*^[43]. Recently, *E. coli* carrying the *iroN* gene, which encodes for a receptor for iron-chelating siderophores, was more frequently isolated from inflammatory and unchanged mucosa of active-phase UC patients^[23].

On the other hand, approximately one decade ago, Darfeuille-Michaud *et al*^[45] discovered a new pathotype of *E. coli* with distinctive phenotypic pathogenic traits that was associated with CD, but not with UC, named adherent invasive *E. coli* (AIEC). Altogether, these observations suggest that specific *E. coli* types could be involved in each IBD. We further discuss this issue in the section dedicated to AIEC prevalence in ulcerative colitis.

ADHERENT INVASIVE *E. COLI*

To date, AIEC is the most likely candidate to cause specific damage to people who are genetically susceptible to the development of CD, and therefore the following sections will focus on discussing the most recent research findings on this pathovar. We review (1) the

latest research regarding AIEC pathogenicity; (2) the prevalence and abundance of the pathotype in several intestinal disorders, discussing its putative contribution to other intestinal diseases in addition to CD; (3) the evidence that supports a lack of host-specificity and thus a risk for zoonosis; and (4) recent research that points to a putative role for environmental factors in the fate of AIEC development in the intestine.

Definition

The AIEC pathotype was defined as *E. coli* strains that (1) are able to adhere to differentiated Caco-2 and/or undifferentiated I-407 intestinal epithelial cells with an adhesion index equal or superior to 1 bacteria per cell; (2) are able to invade I-407 cells with an invasion index equal or superior to 0.1% of the original inoculum; (3) involve host cell actin polymerization and microtubule recruitment in bacterial uptake; (4) do not have known invasive determinants; and (5) are able to survive and replicate within J774-A1 macrophages^[45]. Since its definition, invasive determinants characteristic from ExPEC have been detected in some AIEC, but not consistently in all AIEC, and thus are not a particularity of the AIEC pathotype^[6,13,36,46-48].

Molecular basis of AIEC pathogenicity

Pathogenicity mechanisms of AIEC have mainly been studied in the reference AIEC strain LF82, and its features have been comprehensively linked to many characteristics of CD pathogenesis.

Adhesion to intestinal epithelial cells is in part mediated by type 1 pili, which interact with the glycoprotein CEACAM6 in a mannose-associated manner^[49,50]. CEACAM6 is overexpressed in CD patients with ileal disease, which makes them more susceptible to overcolonization by AIEC. Although type 1 pili is present in almost all *E. coli*, including non-pathogenic strains, we have recently demonstrated that AIEC strains usually present FimH adhesin variants that allow them to more efficiently bind intestinal epithelial cells^[31]. Some non-AIEC strains carry these mutations as well, but they do not express type 1 pili. Flagella are also important for adhesion to and invasion of intestinal epithelial cells and elicit the secretion of the pro-inflammatory cytokine IL-8 and chemokine CCL20 in polarized intestinal epithelial cells, which in turn leads to the recruitment of macrophages and dendritic cells to the site of infection^[51,52]. The further secretion of INF γ and TNF α by macrophages and lymphocytes leads to CEACAM6 expression, which enhances AIEC colonization. The binding of LF82 type 1 pili to CEACAM6 and flagella to TLR5 in intestinal epithelial cells induces the production of HIF-1 α and activation of the classical NF- κ B pathway^[53]. In turn, these molecules cooperatively control the transcription of IL-8 and pro-angiogenic factors contributing to inflammation and vascularization.

The intermediate filament vimentin, expressed on the host cell surface of mesenchymal cells, has been re-

cently proposed to act as a receptor for AIEC^[54]. At the intracellular side, vimentin leucine-rich repeats interact with NOD2 leading to the recruitment of these proteins at the plasma membrane. This is necessary for a proper function of NOD2 in terms of antigen detection, NF- κ B activation and autophagy induction. CD patients have specific NOD2 variants (L1007fs and R702W) that are unable to interact with vimentin and, in turn, they localize in the cytosol. That leads to a defective inflammatory response, autophagy induction and handling of CD-associated AIEC. Altogether, NOD2 and vimentin appear to play an important role in AIEC recognition and polymorphisms in these two proteins may have an impact on the ability of AIEC to colonize the host.

A new host-microbe interaction that mediates adhesion of LF82 to intestinal epithelial cells and involves a bacterial and a human chitinase has recently been proposed^[55]. Chitinases are enzymes that hydrolyze chitin, a long-chain polymer of an N-acetylglucosamine. The authors demonstrate that specific polymorphisms in two chitin binding domains characteristic of LF82 and other pathogenic *E. coli* are required to interact with an N-glycosylated asparagine of the human chitinase CHI3L1. Interestingly, human chitinases are overexpressed in intestinal epithelial cells and moderately expressed in cells of the lamina propria during inflammation.

Outer membrane vesicles (OMVs) containing the transmembrane protein OmpA play a role in LF82 invasion of intestinal epithelial cells^[48]. OmpA binds the endoplasmic reticulum-localized stress response chaperone Gp96 that is overexpressed on the apical surface of ileal epithelial cells in patients with CD. OMVs fuse with host cells, and it is thought that the release of bacterial effectors that are still undefined is involved in the actin polymerization and microtubule recruitment that occurs during invasion. Point mutations in the *ompA* sequence of LF82 and other B2 strains mediate better interactions with Gp96^[56]. In turn, Gp96 is overexpressed in the ileum of CD patients, which renders them more susceptible to AIEC infection.

Once inside the host cell, LF82 bacteria can be found in several types of intracellular compartments: individually or in groups within single membrane vacuoles, within damaged vacuoles, or within LC3-positive autophagosomes, which indicates that autophagy restricts a subpopulation of intracellular LF82 bacteria^[57]. Nevertheless, it was recently demonstrated that AIEC can abrogate the autophagic process^[58]. Intracellular LF82 activates NF- κ B, leading to the increased expression of MIR30C and MIR130A in T84 cells and in mouse enterocytes, and the upregulation of these microRNAs reduces levels of ATG5 and ATG16L1, inhibiting autophagy and enhancing the inflammatory response. In turn, defects in autophagy mechanisms related to the ATG16L1 and IRGM genes have been associated with CD patients, and these defects confer an advantage for AIEC to survive inside human cells^[57]. Therefore, it is a combination of host deficiency factors and AIEC

pathogenicity that determines the fate of intracellular *E. coli* survival.

In addition to adhesion and invasion capacity, LF82 is also able of translocating *via* the M cells of the Peyer's patches, gaining access to the lamina propria. This interaction is mediated by type 1 pili and long polar fimbriae (Lpf), which can interact independently with GP2, a surface protein specific to M cells. It is of note that the sites of initial inflammation in CD are the Peyer's patches and colonic lymphoid follicles; thus, this mechanism of translocation is consistent with early clinical signs of the disease^[59].

Another mechanism that can facilitate bacterial translocation is the ability of LF82 to alter intestinal permeability by inducing the expression of the pore-forming protein claudin-2^[60] and by displacing ZO-1 and E-cadherin from apical tight junctions, leading to decreased transepithelial resistance and loss of barrier function^[17,61]. Besides, pro-inflammatory cytokines like TNF α can drive alterations in intestinal permeability^[62]. As AIEC infection induces the secretion of large amounts of TNF α and IL-8^[17]; thus, the loss of barrier function induced by LF82 can in part be mediated by the induction of TNF α secretion.

A novel mechanism of pathogenicity observed in LF82 and two other AIEC strains (O83:H1 and UM146) is the evasion of host immune responses *via* subversion of the IFN γ pathway in intestinal epithelial cells^[63]. Phosphorylation of the Signal Transducer and Activator of Transcription STAT-1 is blocked, thus preventing the transcription of IFN γ -dependent genes, which reduces host immune responses and results in an inability to mount an appropriate anti-microbiocidal response. Enterohemorrhagic *E. coli* (EHEC) strain O157:H7, in part through its Shiga toxin, is also able to block tyrosine phosphorylation and activation of STAT1 after IFN γ stimulation, in contrast with enteropathogenic *E. coli* E2348/69 or commensal *E. coli* HB101 which do not present this mechanism of pathogenicity. However, AIEC do not present Shiga toxins. Presumably a small secreted peptide may be responsible for this pathogenic mechanism in AIEC^[63].

Once AIEC has gained access to the lamina propria, these bacteria can be engulfed by macrophages. Intramacrophage LF82 do not escape into the cytoplasm but induce the formation of a large vacuole (phagosome) that fuses with lysosomes^[64], suggesting that AIEC bacteria have the ability to replicate in an environment with acidic pH, oxidative stress, active proteolytic enzymes, and antimicrobial compounds. Indeed, it was demonstrated *in vitro* that an acidic environment is necessary for replication of AIEC LF82 bacteria^[64]. The protease HtrA and the thiol-disulfide oxidoreductase DsbA have been reported to be important for survival and replication within macrophages^[65,66]. The authors linked these proteins to the ability of LF82 to resist the stress conditions of the phagolysosomes, as isogenic mutants for these proteins were less efficient in growing in acidic and

nutrient-poor medium, and these proteins were overexpressed not only in LF82 during macrophage infection but also in acidic nutrient-poor medium. Interestingly, the overexpression of HtrA is dependent on the LF82 background, as non-pathogenic *E. coli* do not overexpress that protein under similar growth conditions. The RNA-binding protein Hfq, which functions as a global posttranscriptional regulator of gene expression, has also been implicated in survival and replication within macrophages and in stress tolerance but also other aspects of LF82 pathogenicity, such as adhesion and invasion capability^[67]. Hfq binds small regulatory RNA molecules, facilitating their interaction with mRNA, but the target genes are still unknown.

Continuous replication of LF82 within macrophages results in the secretion of high levels of TNF α without inducing host cell death^[68]. This can explain inflammation and granuloma formation in the gut of CD patients, which has been demonstrated *in vitro*^[50,69,70]. A direct role for LF82 in delaying apoptosis of infected macrophages and dendritic cells has recently been reported^[71]. LF82 infection was found to alter the function of caspase-3, a protease that plays an essential role in apoptosis, and to increase degradation of this molecule in the proteasome.

Also supporting AIEC capability to replicate within immune cells, strain LF82 was able to replicate within monocytes isolated from CD patients for the first 20 h after infection but then CD monocytes started to clear intracellular bacteria^[72]. Interestingly, those patients with polymorphisms in *CARD15* gene (R702W, G908R and 1007fs) showed reduced early inflammatory response towards AIEC infection with decreased levels of IL-1 β , IL-6 and IL-10. In contrast, Asp299Gly mutation in TLR4 had no effect on monocyte response to AIEC. Besides, a recent study revealed that CD monocyte-derived dendritic cells stimulated with lipopolysaccharide show an attenuated inflammatory response with decreased levels of IL-6 and IL-1 β , as well as an impaired autophagy with reduced LC3 expression^[73]. Moreover, these cells had a reduced capacity to support the expansion of allogeneic Th17 cells from CD4+ memory T cells. The authors propose that mucosal Th17 activation in CD patients is a secondary event in response of poor bacterial clearance due to defects in innate immunity. Further studies showing AIEC effects on CD defective dendritic cells regarding, not only cytokine release, but also autophagy function and the level of IL-17A response induction in T cells, are necessary to decipher whether the alterations observed in lipopolysaccharide-stimulated dendritic cells equally occur after AIEC exposure.

AIEC LF82 bacteria are also able to invade and replicate within human neutrophils, but in contrast to its behavior inside macrophages and intestinal epithelial cells, LF82 induces the autophagic death of infected neutrophils, which later undergo an alternative cell death process called NETosis^[74]. In neutrophils, LF82 are localized inside autolysosomes, as observed by the colocalization of phagosome and lysosome markers, but there is no

acidification, which suggests that LF82 avoids autolysosome maturation. Infected neutrophils secrete cytokines, in particular IL-8, contributing to mucosal inflammation.

The ability to form biofilms is a pathogenic feature frequently found among AIEC strains. We found that 17 out of 27 AIEC strains and only 9 out of 38 intestinal non-AIEC strains were biofilm producers^[75]. Motility and flagellar type are of relevance in biofilm production, as non-motile strains were not able to form biofilms, and all strains with the H1 flagellar antigen were strong biofilm producers. Recently, Chassaing *et al.*^[76] have demonstrated the ability of the LF82 strain to form biofilms on intestinal epithelial cells using cell culture and animal models.

Genetic factors characteristic of the AIEC pathotype

Despite all the research conducted on AIEC pathogenicity, we still do not know the genetic factors that are characteristic of the AIEC pathotype. The majority of genes related to its pathogenicity are not AIEC-specific, as is the case for *fimH*, *ompA*, *dsbA* or *btrA*, and are present in the majority of *E. coli* strains, including non-pathogenic strains^[31,48,65,66]. Point mutations or differential gene expression are involved in the increased fitness and/or virulence of AIEC strains. Unfortunately, these genetic factors have been studied in very few strains or exclusively in the prototype strain LF82. Conversely, virulence genes that are not usually present in non-pathogenic *E. coli*, such as *afaC*, *pks* or *lpf*, have been found frequently, but not consistently, in AIEC strains^[13,59,77]. AIEC strains are clonally diverse, belong to different serotypes and carry different sets of virulence genes that are characteristic of ExPEC strains; these features also describe non-AIEC ExPEC-like strains inhabiting the intestinal mucosa^[13]. The AIEC pathotype comprises high genotype variability, which complicates the identification of specific genetic factors of the pathotype.

It is of note that despite the genetic similarity between AIEC and ExPEC, the latter generally does not exhibit the AIEC phenotype. We determined that only 4 out of 63 ExPEC strains of different origins were AIEC-like^[78], conferring a particular identity on the pathotype. Identification of additional genetic elements or the differential expression of key genes that must be involved in AIEC pathogenicity represents an important milestone that can be achieved through genome- and transcriptome-based studies.

Four AIEC genomes belonging to B2 strains have been sequenced and published to date^[46,47,79,80], and comparative genomics have been carried out for the LF82 and NRG857c strains^[47]. Although novel virulence factors not previously found in AIEC by PCR genotyping, such as a type-6 secretion system, have been detected in genomic islands of the sequenced strains, genomic studies have corroborated the notion that AIEC resembles ExPEC. Unique sequences for AIEC were found in common between the LF82 and NRG857 strains. However, both strains belong to the same phylogroup and

serotype (B2 O83:H1), which indicates they are genetically very close. Given the high variability of AIEC seropathotypes, studying the distribution of these genes in other AIEC strains is essential to confirm whether these elements are common features of the pathotype or are strain-specific. Comparative genomics of phylogenetically distant AIEC strains would presumably reveal a significantly greater number of genetic differences. Although it will complicate the situation, sequencing additional AIEC strains from different phylogenetic origins is crucial to determine the common genetic features involved in the AIEC phenotype.

AIEC localization in the intestinal mucosa

AIEC have generally been isolated from tissue samples, but there is no evidence regarding its exact localization within the intestinal mucosa. Although AIEC are invasive bacteria, they have not been convincingly observed within intestinal epithelial cells or in the lamina propria in resected tissue or mucosal biopsies. The studies conducted by Martin *et al.*^[12] and Elliott *et al.*^[36] addressed whether *E. coli* are intraepithelial or mucosa-associated by treating biopsies with gentamicin. This approach has brought indirect evidence of *E. coli* invasion of intestinal epithelial cells in CD but not in UC. However, the complete AIEC phenotype was not studied in the intracellular *E. coli* strains obtained from these studies.

Currently, identifying the exact localization of AIEC strains in the mucosa is nearly impossible to do, as no molecular tools that specifically target the AIEC pathovar are available. Some evidence has been obtained using animal models infected with known reference strains. For example, by staining for the O83 antigen, it has recently been demonstrated that the LF82 and NRG857c strains colonize the ileum, cecum and colon of several mouse models and that they are located at the base of the crypts and within goblet cells^[81]. Engineered LF82 with a plasmid containing the GFP protein permitted fluorescence-microscopic examination of the localization of LF82 in the nematode *C. elegans*. In this situation, there was robust gut colonization, but bacteria remained in the lumen and were not attached to intestinal epithelial cells^[67]. To visualize the extent of bacterial adhesion and invasion in *in vivo* infection, Low *et al.*^[55] stained *E. coli* lipopolysaccharides with specific antibodies and compared basal levels of fluorescence in uninfected mice (corresponding to indigenous bacteria) with levels in infected mice. They found higher counts of stained bacteria in the intestinal epithelial cells and lamina propria of infected mice, suggesting AIEC intestinal epithelial cell invasion and translocation.

Pathobiont rather than a true pathogen

Despite the virulence genes that are encoded in the genome of many AIEC strains and the mechanisms of pathogenicity reported for the prototype strain LF82, AIEC are generally considered pathobionts. This assumption is supported by the fact that, although at a

lower frequency than in CD, healthy subjects can carry AIEC in their intestinal mucosa^[6,13,17,45,82]. The prevalence varies between studies, ranging from the absence to 15.8% of colonic samples with AIEC and from 6.2% to 18% in ileal samples. Although AIEC bacteria may colonize the intestinal mucosa of non-IBD patients, these bacteria usually do not translocate a healthy mucosal barrier, as bacterial invasion of the mucosa has not frequently been observed in control patients^[14] and intracellular *E. coli* was rarely cultivated from the colonic mucosa of healthy subjects (from the absence to 9%)^[6,26,83]. AIEC strains are more abundant, and consequently more frequently found, in the ileum than in the colon of healthy subjects. We found that AIEC accounted for 3.58% and 0.95%, respectively, of the ileal and colonic *E. coli* populations^[13]. Accordingly, a larger number of AIEC LF82 bacteria were attached to ileal than to colon tissue in *ex vivo* samples from healthy subjects infected with this AIEC strain^[84]. Altogether, these data suggest that AIEC can more easily colonize the ileum with respect to other *E. coli*, and at least approximately 1 out of 6 healthy individuals can be considered “asymptomatic carriers”.

Genetic or environment-derived host defects at the intestinal barrier may determine the ability of AIEC to colonize and translocate the gut. A number of host deficiencies frequently found in CD patients have been linked with the increased ability of AIEC LF82 to cause infection. For example, these defects include the overexpression of the CEACAM6 and Gp96 receptors in the apical membrane of intestinal epithelial cells, which facilitates AIEC adhesion and invasion^[48,49], or defects in autophagy related to NOD2, ATG16L1 and IRGM function and expression, which impair the ability of host cells to resolve infections^[57,85]. Additionally, it has been suggested that the altered bile salts metabolism in CD patients could enhance the expression of long polar fimbriae in AIEC, which could permit better translocation *via* M cells^[86]. Moreover, decreased levels of the protease meprin, which are characteristic of severe inflammation in IBD patients, have been proposed to determine the fate of AIEC in terms of their ability to colonize the host, as these proteases degrade type 1 pili^[87].

PREVALENCE AND ABUNDANCE OF AIEC IN IBD

CD

Intraepithelial *E. coli* with adherent and invasive properties were isolated from the sigmoid colon mucosa in 29% of CD patients^[12] and in 90% of CD patients in a cohort composed of ileal, ileocolonic and colonic disease phenotypes^[36]. Differences between studies could be explained by the disease activity status of the cohort of patients, who were mainly in the relapse stage in the latter study.

In the last decade, several independent laboratories

have reported a higher prevalence of AIEC in CD patients than in healthy subjects^[6,13,17,45,82]. Unfortunately, not all of these studies categorized CD patients by their disease subtype or analyzed prevalence based on anatomic location in the gut. The first study was conducted by Darfeuille-Michaud *et al.*^[45] in 2004 and revealed that 22% of CD patients with ileal involvement harbored AIEC strains in ileal chronic lesions and at a similar frequency in healthy mucosa. However, AIEC bacteria were more likely to be found in the early ileal lesions that occurred in patients after ileostomy (36.4%). AIEC strains were only isolated from the colon of 3.7% of CD patients with a colonic disease phenotype. The authors proposed an association between AIEC and ileal CD and suggested that the pathovar could be involved in the initiation of the inflammatory process. Conversely, Baumgart *et al.*^[6] reported a prevalence of AIEC strains in the ileum of 38.5% of CD patients with ileal involvement and 37.5% with colonic CD, indicating that AIEC is associated both with ileal and colonic disease phenotypes. Sasaki *et al.*^[17] demonstrated that 24.3% of CD patients exhibited AIEC strains, but neither the localization of these strains in the gut nor the disease phenotypes of the positive patients were detailed. A similar prevalence was reported by Dogan *et al.*^[82] in the ileum of CD patients with ileal disease. We detected AIEC strains at a higher frequency in comparison with previous studies, most likely due to the methodological approach used. Whereas other studies analyzed from 1 to 15 *E. coli* colonies per patient, we searched for AIEC strains in a collection of 95 - 150 *E. coli* colonies per patient. This approach not only enabled us to obtain a more accurate prevalence value but also to study the abundance of AIEC strains within the *E. coli* population. We detected AIEC strains in the ileum of 54.5% of CD patients and in the colon of 50% of CD patients^[13]. Although data depicted by disease subtype were not reported in the original work, we also found a higher prevalence in CD patients with ileal involvement (66.7% of ileal and 58.3% of colonic samples) than those with colonic disease (50% of ileal and 25% of colonic samples). Colonic CD patients denoted also a high prevalence of AIEC, what supports the observations of Baumgart *et al.*^[6], but the pathotype was more frequently found in the ileum than in the colon of CD patients, in line with the findings of Darfeuille-Michaud *et al.*^[45] The abundance of AIEC, defined as the percentage of AIEC within the *E. coli* population, was low and variable, ranging from 1% to 50%. On average, AIEC isolates represented 9.3%, 3.7% and 3.1% of *E. coli* isolates in ileal, ileocolonic and colonic CD patients, respectively. Jensen *et al.*^[84] supported these data using quantitative PCR targeting indigenous LF82 bacteria. The increased expression of CEACAM6 in the ileum of ileal CD patients may explain the higher prevalence and abundance of AIEC in CD patients with ileal involvement. However, additional host-microbial interactions or environmental factors may be involved in colonization of the colonic mucosa, as no differences

in CEACAM6 expression exist at the level of the colon between CD patients and control subjects^[49]. Our work demonstrates that AIEC are more prevalent than expected in all CD disease subtypes, reinforces the hypothesis that the microenvironment of ileal CD specifically favors AIEC expansion, and suggests that the colon is also a niche effectively colonized by AIEC.

UC

More than two decades ago, the adhesion capabilities of *E. coli* from both UC and CD patients were assessed. Mannose-resistant adhesion was characteristic of *E. coli* from both IBDs, which raised the question of whether adhesive *E. coli* could also be involved in UC pathogenesis^[88,89]. Recent studies have confirmed that, in UC patients, adherent *E. coli* strains are found as frequently as^[90] or even more frequently than^[34,91] in CD patients. An undefined adhesion pattern was most prevalent in *E. coli* from both UC and CD patients^[42], although aggregative adherence was particularly frequent in UC patients^[42,90]. Molecular tools to detect adhesive determinants of IBD *E. coli* did not demonstrate specific adhesion factors in UC *E. coli* in comparison to CD *E. coli*^[10,37,41,42], whereas in other studies UC *E. coli* carried some adhesion factors more frequently than CD *E. coli*^[30,34,44]. Some of these studies are based on pediatric or newly diagnosed patients, which provides supporting arguments for the early contribution of adherent *E. coli* to IBD rather than being its development a consequence of inflammation. Moreover, the higher frequency of *E. coli* B2 strains with at least one positive adhesion-related gene was correlated with disease activity in UC patients (86% in active *vs.* 13% in non-active patents)^[32]. Therefore, there is substantial agreement among studies regarding the adhesion capacity of *E. coli* strains from UC patients.

Intracellular *E. coli* were cultured from 47%^[36] and 19%^[12] of UC patients in two studies using gentamicin protection assay. However, few works have sought to identify the AIEC pathovar in UC patients, and some controversial results have been obtained. In the first study that searched for AIEC in UC any of UC patients had AIEC bacteria in their colon^[45], and similar results were obtained in a later study^[92]. In contrast, in studies with larger cohorts, one of them based on pediatric patients, AIEC were detected in 7.2% to 10% of UC patients^[17,93]. Other investigators that studied the invasion ability of IBD *E. coli*, but did not study the complete AIEC phenotype, detected a high prevalence of invasive strains in UC patients (37.5%)^[44]. Moreover, similar invasion rates in I407 cells were observed for *E. coli* from pediatric UC and CD patients^[42], whereas in a previous study the invasion index using differentiated Caco-2 cells was lower in *E. coli* from UC than CD patients^[17]. Noteworthy, the intra-macrophage survival capacity of *E. coli* strains was found to be highest in UC patients from a cohort of newly diagnosed IBD patients. Unfortunately, no information about adhesion and invasion abilities was

provided^[30].

Sasaki *et al.*^[17] observed that although AIEC from UC were less invasive than CD *E. coli*, they induced the secretion of similar amounts of TNF α and higher amounts of IL-8, suggesting that UC-associated *E. coli* are distinct from those associated with CD. Accordingly, a recent study reported that CD *E. coli* are frequently *lpf+ afaC+*, whereas UC *E. coli* do not possess *lpf* gene and frequently harbor the *afaC* and *pks* genes together^[77]. *Lpf* mediate translocation of bacteria via M cells, while the afimbrial adhesin AfaC mediates a diffuse adherence to and invasion of intestinal epithelial cells and also induces vascular endothelial growth factor expression. The polyketide synthase gene complex (*pks*) contains the genes to synthesize the metabolite colibactin, a genotoxin with the ability to cause epithelial DNA damage.

The evidence collected to date suggests that *E. coli* strains with adhesive and other virulence properties could be involved in UC pathogenesis, but further work clarifying the role of these strains in conjunction with host defects in the mucosal barrier is needed. Furthermore, in view of the few studies and conflicting results regarding AIEC prevalence in UC, additional studies characterizing *E. coli* populations from different anatomical sites, and for both affected and unaffected tissue, in active and inactive UC patients would be of relevance to elucidate the possible role of AIEC in UC.

E. COLI POPULATIONS IN OTHER INTES-TINAL DISEASES: IS AIEC INVOLVED?

Colorectal cancer

An analysis of fecal bacterial diversity by pyrosequencing demonstrated that the *Escherichia/Shigella* genus was enriched in colorectal cancer (CRC) patients^[94]. In contrast, studies conducting quantitative PCR did not find an increase in the *E. coli* population in CRC^[8,91]. However, intracellular *E. coli* has frequently been found in CRC patients. Swidsinski and collaborators detected intracellular *E. coli* in 87% of patients with CRC and not in controls using a gentamicin protection assay^[95]. Similarly, Martin *et al.*^[12] isolated intramucosal *E. coli* from 33% of tumors in CRC patients and 9% of control subjects, surpassing the prevalence found among IBD patients, and Bonnet *et al.*^[96] isolated intramucosal *E. coli* in 86% of colon cancer tumor specimens and 48% of diverticulosis samples. Moreover, high levels of mucosa-associated *E. coli* correlated with poor colorectal carcinoma prognostic factors and a higher proliferative index of epithelial cells, suggesting a role for these bacteria in tumor progression.

E. coli strains isolated from the study by Prorok-Hamon *et al.*^[77] were hemagglutination-positive, adherent to HT29 and I407 intestinal epithelial cells and frequently able to invade I407 cells, all characteristics that resemble the AIEC pathotype. A recent study conducted by the same research group showed that at least one of the isolates obtained from a patient with CRC shared the com-

plete AIEC phenotype. In addition, *E. coli* isolated from a pediatric cohort with polyposis, who were included as a healthy control group, showed the highest invasion efficiency compared with *E. coli* strains isolated from IBD children^[42]. However, as far as we know, there is no data regarding the prevalence of AIEC in patients with CRC.

Several studies have demonstrated that *E. coli* associated with CRC are frequently colibactin-producing^[72,93-95]. Not only is the *pks* genomic island encoding for the genotoxin colibactin frequent in CRC, but other cyclomodulins such as CNF, CDT and Cif. Buc *et al.*^[97] found that cyclomodulin-encoding genes were over-represented among *E. coli* from CRC patients (65.8%), particularly distal colon cancer (76.5%), compared with diverticulosis samples (19.54%). These molecules can be genotoxic and/or modulate cellular differentiation, apoptosis, and proliferation. Prorok-Hamon *et al.*^[77] observed that CRC *E. coli* frequently harbored the *pks* gene but also the adhesins AfaC and LpfA, partially resembling those *E. coli* isolated from CD and UC. These factors confer the ability to adhere to and invade I407 cells, to upregulate vascular endothelial growth factor expression in intestinal epithelial cells, and presumably, to translocate *via* M cells and cause genotoxicity to host cells. Recently, pathogenic cyclomodulin-positive *E. coli* strains were found to be more prevalent in the mucosa of patients with advanced stages of the disease^[96].

Few studies have been focused on *E. coli* populations in CRC patients to date, and the results obtained point to a putative role for a subset of *E. coli* with pathogenic features relevant to CRC pathogenesis. Given that AIEC possessing virulence factors relevant to enterocyte adhesion and invasion, vascular endothelial growth factor expression and carcinogenesis have been detected in CRC patients and the fact that intramucosal *E. coli* with features similar to AIEC have been more frequently found in CRC than in IBD patients, further studies determining the prevalence of AIEC in CRC are needed to corroborate or refute the hypothesis for a putative role for AIEC in CRC.

Coeliac disease

Coeliac disease is a chronic inflammatory disorder exclusively affecting the small intestine, in which genetically predisposed individuals feature a permanent intolerance to dietary gluten. Several studies have provided evidence that coeliac patients exhibit intestinal microbial dysbiosis, similar to what occurs in IBD patients. In a study based on PCR-TGGE of duodenal samples, *E. coli* was found more frequently in coeliac children (92.1%) than in healthy children (20%)^[98]. Quantification of *E. coli* by FISH showed also that this species was more abundant in active coeliac patients than in inactive patients and controls^[99], but this was not observed in fecal samples^[100]. Another study found changes in *Enterobacteriaceae* diversity and increased virulence-gene carriage in *E. coli* isolates from coeliac children^[101]. In particular, *E. coli* strains largely belonging to the B2 and D phyloge-

netic groups and carrying ExPEC-like features, *e.g.*, pilus P and hemolysin A, were found to be more abundant in coeliac patients when compared to healthy controls. This dysbiosis of the *E. coli* population is similar to that found in CD patients.

Given the association between *E. coli* and coeliac disease in terms of abundance and the correlation with disease activity, as well as the genetic similarities between isolates from the intestinal mucosa of coeliac patients and CD patients, further studies aimed at identifying the AIEC phenotype amongst coeliac *E. coli* isolates are of interest to better define the disease specificity of the AIEC pathotype.

ADHERENT-INVASIVE *E. COLI* IN ANIMALS WITH INTESTINAL DISEASE

AIEC strains isolated from CD patients genetically resemble avian pathogenic *E. coli* and other animal ExPEC. We studied the AIEC phenotype in a strain collection obtained from animals with extraintestinal infection and intestinal disease to determine the disease and host specificity of the AIEC pathotype. All these strains were classified as ExPEC in terms of their phylogenetic origin and virulence genotype. ExPEC strains of extraintestinal origin rarely shared the AIEC phenotype, whereas ExPEC-like strains of intestinal origin were frequently AIEC-like in cats (82%), dogs (35%) and swine (32%) with intestinal disease^[102]. The high prevalence of AIEC in companion and farm animals highlights a putative risk of zoonosis between humans and animals. In a previous study, Simpson *et al.*^[103] detected AIEC in boxer dogs. Interestingly, these dogs suffered from granulomatous colitis, a disease with pathological features that overlap with CD, which supports the role of AIEC in human CD and analogous diseases in animals.

Altogether, these results suggest that the AIEC pathotype is disease-specific rather than host-specific and raises the question of whether there is a possible route of transmission between animals and humans. Further studies examining the distribution of AIEC strains in different healthy and diseased animals and in the environment would contribute to our understanding of the epidemiology, putative reservoirs, host-specificity and possible routes of transmission of AIEC.

ENVIRONMENTAL FACTORS INVOLVED IN THE SUCCESSFUL COLONIZATION OF AIEC

Recent studies have implicated some emulsifiers and food stabilizers frequently used in developed countries as having a role in AIEC colonization. Maltodextrin, a polysaccharide derived from starch hydrolysis that is used as food additive, has been shown to markedly enhance AIEC biofilm formation and adhesion to intestinal epithelial cells and macrophages^[104]. Maltodextrin fa-

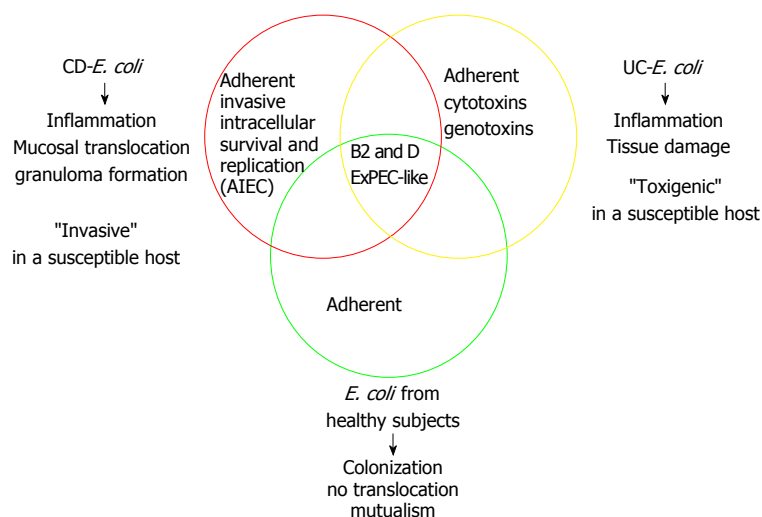


Figure 1 Features of inflammatory bowel disease-associated *Escherichia coli* and impact of this species on Crohn's disease and ulcerative colitis.

vors type 1 pili expression, which is required for biofilm formation and adhesion. Moreover, a higher prevalence of the gene *malX*, which is essential for maltodextrin metabolism, was found in bacteria isolated from ileal CD patients than from healthy controls (71% *vs* 18%, respectively). These observations suggest that a diet rich in maltodextrin would aid maltodextrin-utilizing bacteria, would enhance *E. coli* gut colonization, and thus contribute to dysbiosis. Furthermore, polysorbate-80, an emulsifier commonly used in processed foods, was found to enhance translocation of the AIEC HM605 strain across M cells and intestinal epithelial cells^[105]. Using animal models, we also observed that a diet enriched in fat and sugar induced dysbiosis and low grade-inflammation^[106]. In this work we also showed that dysbiosis and low-grade inflammation in susceptible individuals lead to increased AIEC colonization, what in turn exacerbated the inflammatory response and epithelial barrier disruption.

The type of dietary fiber intake may influence bile acid metabolism. For example, daily dietary supplementation for four weeks with the purified fiber components pectin and cellulose in humans leads to differential bile acid composition. In cellulose-treated volunteers, cholic acid increased whereas deoxycholic acid decreased, which inversely occurred in pectin-treated individuals^[107]. Increased concentrations of cholic acid and chenodeoxycholic acid have been reported in CD patients^[108], and lithocholic acid has been reported particularly in ileal CD patients^[109]. Interestingly, all of these bile salts induced the expression of the *hpf* operon in AIEC LF82 strain^[86]. Therefore, dietary fiber consumption could also influence the tropism of AIEC for CD ileal tissue by altering bile acid composition and thus the expression of *hpf* in AIEC in the gut.

These studies demonstrate that dietary components may impact the success of AIEC in colonizing the host and therefore contribute to disease susceptibility. For that reason, intervention studies are needed to evaluate the effects of diet, probiotics, and/or prebiotics on the intestinal microbial community, including the AIEC population with respect to CD activity status and disease

progression.

AIEC, A CAUSE AND A CONSEQUENCE OF INFLAMMATION

Several studies based on animal models have shown that there is a need of microbial dysbiosis and/or intestinal inflammation to succeed with AIEC infection. An effective colonization only occurs in mice that have been treated with antibiotics^[50,81,110], dextran sodium sulfate^[69] or high-fat/high-sugar diet^[106] before infection, having these treatments an effect on gut bacteria composition and mucosal homeostasis. Moreover, Craven *et al*^[111], nicely showed that moderate to severe ileitis produced by protozoan infection in mice models induced dysbiosis and proliferation of endogenous mucosally invasive *E. coli*. These works suggest that inflammation and dysbiosis favors AIEC proliferation. Therefore, AIEC overgrowth in the intestine can be seen as a consequence of inflammation.

On the other hand, it has been recently shown that AIEC infection itself induced lasting changes in the intestinal microbiota^[112]. This study was conducted on mice lacking flagellin receptor TLR5 (T5KO) which are prone to develop spontaneous colitis. The authors hypothesized that transient colonization of T5KO mice by AIEC results in an altered gut microbiota community with greater proinflammatory potential, which can persist in the host and induce chronic inflammation due to its increased levels of lipopolysaccharide and flagellin. The effects of AIEC infection on host mucosal immunity, barrier integrity and inflammation induction have been demonstrated in multiple animal models^[50,60,69,81,106,110] but the work of Chassaing *et al*^[112] is the first showing that AIEC infection contribute to intestinal dysbiosis. Overall, these studies suggest that AIEC overgrowth in the intestine can be seen as a cause of inflammation.

Therefore, inflammation can instigate imbalances in *E. coli*, especially the AIEC pathotype and, in turn, these bacteria can be involved in a further dysbiosis and in-

creased intestinal inflammation.

CONCLUSION

Substantial evidence indicates that *E. coli* is involved in CD and growing data suggest that this species is also a contributing factor in UC pathogenesis (Figure 1). Studies focused on defining virulence gene profiles of *E. coli* populations have shown that *E. coli* associated to the mucosa of healthy subjects resemble those of IBD patients. Genes related with adhesion, iron transport, capsule formation and toxins are present in *E. coli* from both healthy subjects and IBD patients. These features are thought to be necessary for an effective colonization of the intestinal tract. However, the intestinal microenvironment in IBD patients, especially those in relapse, would predispose to *E. coli* proliferation. Moreover, *E. coli* from CD patients have probably evolved towards the AIEC pathotype, which has the capacity to adhere to and to invade intestinal epithelial cells, as well as to survive and replicate within a number of cell types. Virulence properties of AIEC described to date can explain several features of CD pathophysiology such as inflammation, mucosal bacterial translocation and granuloma formation. Conversely, *E. coli* strains from UC patients appear to present a “toxigenic” behavior rather than the “invasive” pathogenic mechanism of CD- *E. coli*. Recent research has pointed out that *E. coli* from UC patients frequently carry virulence genes related to cytotoxicity and genotoxicity, which can contribute to mucosal inflammation and tissue damage. This is in accordance with previous works that did not found *E. coli* translocating the epithelial barrier of UC patients, and could be linked with some aspects of UC pathophysiology.

Since the AIEC pathotype was defined one decade ago, substantial research has been conducted focusing on the identification of the mechanisms of pathogenicity and also in the field of epidemiology with regard to CD. However, additional epidemiologic studies are still needed to corroborate the role of AIEC in CD and to clarify the AIEC disease- and host-specificity. An important limitation to epidemiological studies is the absence of specific molecular tools to detect and quantify this pathotype, as the current available techniques to identify the AIEC pathotype are based exclusively on phenotypic screening of cultured bacteria, which is highly time-consuming. The execution of large-scale epidemiologic studies would also provide new insights into its distribution, putative reservoirs and transmission pathways. Moreover, the molecular bases of AIEC pathogenicity are still not fully understood, as only a few model strains have been studied and there is a wide variety of seropathotypes and phylotypes within the AIEC pathotype. Genomic and transcriptomic studies including wider and more diverse AIEC strain collections could assist in identifying new genetic elements associated with the AIEC phenotype, which may help us to gain a better understanding of the mechanisms of pathogenicity and

could result in significant advances in the detection of new therapeutic targets for CD.

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Similarities and differences between Behçet's disease and Crohn's disease

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and different clinical findings, however both diseases show intestinal inflammation. The differential diagnosis may be difficult when the symptoms of the two disease processes are very similar. This review focuses on the similar and different characteristics of Behçet's disease and Crohn's disease.

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Abstract

Behçet's disease (BD) is a chronic inflammatory condition with multisystem involvement. Approximately 10%-15% of patients present with gastrointestinal involvement. Involved sites and the endoscopic view usually resemble Crohn's disease (CD). In addition to intestinal involvement, oral mucosa, the eyes, skin, and joints are commonly affected. No pathognomonic laboratory test is available for the diagnosis of either disease. Management approaches are also similar in various aspects. Differentiating BD from CD is highly challenging. In this article, the similarities and differences between BD and CD in terms of epidemiology, etiopathogenesis, clinical and imaging findings, and histopathological and therapeutic approaches are reviewed.

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Key words: Behçet's disease; Crohn's disease

Core tip: Behçet's disease and Crohn's disease are chronic inflammatory conditions caused by lesions similar to those seen in the bowels. There are similar

INTRODUCTION

Behçet's disease (BD), which was first defined by Hulusi Behçet, a Turkish dermatologist, in 1937, is a chronic inflammatory disease with multisystem involvement^[1]. It presents with remission and exacerbation of mucocutaneous, ocular, articular, vascular, or gastrointestinal lesions. Crohn's disease (CD), on the other hand, is a chronic relapsing inflammatory disorder of the gastrointestinal tract, presenting with BD-like extra-intestinal manifestations^[2]. Both of these chronic immune-mediated inflammatory disorders are likely to affect patients at a younger age accompanied by fluctuating courses. It is possible that a patient with CD meets the criteria for BD. The differential diagnosis in some cases is quite difficult, particularly in the presence of gastrointestinal involvement. Differentiation is usually based on the involvement of different organs. This review aims to investigate the similar and different characteristics of BD and CD.

EPIDEMIOLOGY

The prevalence of BD varies geographically and the

disease is more prevalent in certain groups. It is most common in populations clustered along the ancient Silk Road. Turkey has the highest prevalence (80-370 cases/100000), followed by Asia and the Middle Eastern countries, including Israel, Saudi Arabia and Iran. BD can be seen in all countries worldwide due to immigration^[3-6]. The age at onset of the disease is usually between 20.8 and 40 years, as it is more common in young individuals^[3]. Patients aged 16 years with initial symptoms, considered as childhood-onset BD, have also been reported. The male-to-female ratio varies regionally. The disease is more common among men in Russia, Saudi Arabia, Iraq, Lebanon, Jordan, Kuwait, Greece, Italy, Turkey, and Iran, while it is more frequent among women in Japan, South Korea, and Israel^[3,7].

The incidence of CD may also vary regionally. The incidence of the disease is highest in the United Kingdom, North America, and the northern part of Europe. The prevalence of CD was found to be 133/100000 in the state of Minnesota, United States in 1991. In recent years, several studies showing an increasing incidence ratio have been reported. The incidence is highest in young individuals aged 15 to 29 years. The prevalence of the disease is similar in men and women (the male/female ratio is 2.9-0.76/1)^[8-14].

GENETIC FACTORS

There are familial BD cases in the literature, suggesting that genetic factors play a role in the pathogenesis of the disease. The ratio of familial cases is between 0 and 18.2%. The genetic association between the HLA-B51 gene and BD was first reported in 1982 by Ohno^[15]. This association has been confirmed in many different ethnic groups. The HLA-B5 gene, particularly the HLA-B5101 allele gene, may be a strong candidate locus responsible for the development of BD and HLA-B51 itself may be the major disease susceptibility gene for BD^[16]. It is more likely that the HLA-B51 gene is directly involved in the hyperactivity of neutrophils. Increased neutrophil function has also been reported in HLA-B51-positive BD patients^[17,18].

Familial aggregations and a high degree of disease concordance in twins with CD have been recognized for quite some time. The concordance rate has been reported to be 3% in dizygotic twins and up to 35% in monozygotic twins^[2]. Recent studies have provided an insight into genetic disorders responsible for susceptibility of the disease. Furthermore, these studies have strengthened the evidence that major cytokines, cytokine receptors and cell types are involved in the underlying pathogenesis of the disease. Nucleotide oligomerization domain 2 (NOD2) is the major susceptibility gene for CD. Genome-wide association studies have demonstrated a number of susceptibility genes where NOD2 is encoded. The nucleotide oligomerization domain 2 gene is located at the CD susceptibility locus on chromosome 16q12^[19,20].

PATHOGENESIS

Immunosuppressive agents, which are used in the management of autoimmune disorders, are highly effective in BD, and the role of autoimmunity has been widely discussed in the pathogenesis of the disease^[21,22]. However, anti-nuclear antibody (ANA) positivity, anti-Ro, and anti-La antibodies, which are usually found in autoimmune disorders, have not been found in BD. Several studies have demonstrated the presence of anti-endothelial antibodies, anti-lymphocytic antibodies, and heat-shock protein 60 (HSP60) in BD; however, these antibodies have not been strongly associated with the disease^[23]. Additionally, major histocompatibility complex (MHC) Class II molecules have been associated with autoimmunity. However, BD is strongly associated with HLA-B5, a MHC Class I antigen.

BD is likely to be an autoinflammatory disease, as it presents with mucocutaneous lesions and episodic arthritis without deformity with a very strong acute phase response during these episodes. In BD, neutrophils are implicated in the inflammatory process of natural immune system-mediated disease (caspase pathway, IL-1, IL-18) similar to autoinflammatory diseases^[21]. Mediterranean fever (MEFV) gene mutations, which are the main causes of familial Mediterranean fever (FMF), an autoinflammatory disease, are frequently found in BD^[24,25]. However, the presence of clinical manifestations including uveitis, vasculitis, and thrombosis, which are not seen in autoinflammatory diseases, and the absence of serositis, a very common pathology in autoinflammatory diseases, does not suggest its autoinflammatory nature. Thus, currently, BD is considered to be neither an autoinflammatory nor an autoimmune disorder^[21].

Furthermore, large and small vessel vasculitides may be present in BD. Thrombotic occlusions of the venous branches and aneurysm formations in the arterial vessels may develop. Arterial involvement may lead to bleeding and organ failure, and ultimately death. Immunosuppressive therapies can be effective in the resolution of vasculitis^[26]. Vasculitis-related alterations have been observed in biopsy specimens of oral aphthae, genital ulcers, and skin lesions^[27]. As vasculitis is considered to be a major component involved in the pathogenesis of BD, it is recommended that the disease should be evaluated under systemic vasculitides^[28].

Several microorganisms of the oral microbial flora have been indicated in the pathogenesis of BD^[29,30]. Atypical streptococcal colonization is increased in the oral mucosa. A hyperimmune activity against *Streptococci* has been shown in various studies. *Streptococcus sanguinis* causes increased interleukin-6 (IL-6) and interferon gamma (IFN- γ) secretions in the peripheral blood T-cells^[31]. *Escherichia coli* and *Staphylococcaceae* species have been reported to increase inflammatory cytokines in BD patients. There are also studies showing regression of BD lesions with antibiotherapy in the literature^[32].

Microorganisms that are involved in normal colon

microflora with a mutual relationship with the immune system are also considered to play a role in the underlying pathogenesis of CD. Several products that are produced by these microorganisms such as butyrate and propionate contribute to intestinal inflammation, by affecting immune system cells and cytokines in patients with genetic susceptibility to CD. Reduced mucin production in epithelial cells of the intestinal mucosa is another possible culprit. Genome-wide association studies have revealed a relationship between gene mutations in mucin expression (MUC1, MUC19 and PTGER4) and CD^[2,19].

Innate immune system cells are mostly implicated in the immunopathogenesis of CD^[2]. Pattern recognition receptors such as Toll-like receptors (TLR) and nucleotide binding domain (NOD) like receptors (NLR) have a critical role in the recognition of the molecular patterns of innate immune system cell pathogens. There is a strong association between NOD2/CARD15 polymorphisms and CD. NOD2/CARD15 encodes an intracellular receptor that is expressed predominantly in monocytes and Paneth cells. These pattern receptors are substantially expressed by dendritic cells lying beneath the intestinal epithelium. Dendritic cells may have reduced regulatory T-cell stimulation, which leads to immune tolerance in CD. These cells are responsible for organization of the relationship between microbial products and immune system cells, and identify immunity or tolerance development. The inflammasome complex of the lamina propria, which is implicated in mononuclear cells, is crucial for the immune response. The stimulation of NLPR3, caspase-1, and pro-interleukin-1 causes a significant increase in pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-alpha) and interleukin (IL)-6. IL-17, IL-23, and IL-27 are crucial players in inflammatory alterations during the disease process^[19,33]. Some authorities have adopted CD as an autoinflammatory disease due to its potent inflammation pathways^[34]. Unlike patients with BD, the incidence of MEFV gene mutations remains unchanged in patients with CD^[35]. The ANA and anti-neutrophil cytoplasmic antibodies (ANCA) positivity is higher in the patient population than healthy individuals. Nearly 40% to 70% of patients also have positive anti-*Saccharomyces cerevisiae* antibodies (ASCA), which are associated with disease severity. The ASCA positivity is higher in patients with CD than BD^[36-38].

CLINICAL MANIFESTATIONS

Extra-intestinal manifestations

BD is a multisystem condition usually presenting with oral mucosa, ocular, articular, and vascular involvement. Gastrointestinal, neurological, and cardiac involvement are relatively infrequent. Nearly all patients suffer from recurrent oral ulcers. These ulcers are classified as large, small, or herpetiform, based on their size. They are extremely painful and may involve the buccal mucosa,

labial mucosa, tongue, the soft and hard palate, and the pharynx. The incidence of genital ulcers with scar formation is relatively low compared with oral ulcers. These painful ulcers are quite similar to oral ulcers in appearance. They may be found in the scrotum and penis in men, and the vulva, vagina, and cervix in women. Additionally, nearly two-thirds of patients with BD have skin changes including acne-like papules, pustules, pseudofolliculitis, and erythema nodosum-like lesions. Due to superficial thrombophlebitis, shifting, and painful subcutaneous nodules can be palpable^[7,39-43].

The pathergy phenomenon is a hyper-reactive response to minor trauma. The test is based on the principle of using a 21-25-gauge needle inserted into the skin. Positive test results show papulopustular lesions in the skin or erythematous reactions of the surrounding tissue within 24-48 h. The positive predictive value of the pathergy test varies regionally as the rate of positive pathergy test differs in different countries, and is highest in countries along the ancient Silk Road (30%-70%). The diagnostic value of diagnostic criteria is reduced when pathergy positivity is excluded. In addition, the pathergy test, which involves intradermal monosodium urate crystals, is more sensitive^[7,43].

Ocular involvement in BD includes anterior or posterior uveitis, vitritis, retinal vasculitis, retinal vein thrombosis, corneal ulcers, and retrobulbar neuritis. Ocular disease may be the initial manifestation of the disease. BD-associated uveitis is defined as chronic and recurrent non-granulomatous panuveitis and retinal vasculitis with a bilateral course. The disease usually presents with acute inflammatory episodes that resolve within days or weeks. Recurrent episodes may result in permanent vision loss. Furthermore, as uveitis is rarely accompanied by conjunctivitis, scleritis, episcleritis, or sicca syndrome, other conditions should be suspected in patients with ocular involvement^[40-44].

Musculoskeletal disorders are also common in patients with BD. Palindromic asymmetric arthritic exacerbations involving the knee, wrist, and ankle may develop. Chronic erosive arthritis is relatively rare. The incidence of sacroiliitis has been reported to increase in patients with BD. Due to peripheral arthritis characteristics and sacroiliac joint involvement, BD is evaluated in the spectrum of seronegative spondyloarthropathy. An overlap of relapsing polychondritis and BD, known as mouth and genital ulcers with inflamed cartilage (MAGIC) syndrome, may also develop in patients with cartilaginous inflammation^[42-45].

BD-related vasculopathy differs from other vasculitides, due to its pattern of arterial and venous involvement. Venous thrombus may develop. It may present with superficial thrombophlebitis or involve deep veins, as well as the inferior/superior vena cava, the right atrium, or intracranial large sinuses. The major hepatobiliary disease is Budd-Chiari syndrome, which is one of the leading causes of mortality^[46]. Unlike other thrombotic events, embolism is not anticipated. Primary thrombosis,

which is often accompanied by right atrial thrombi, may occur in the pulmonary artery and its thin branches. In addition, arterial aneurysms are common. Pulmonary artery aneurysms may lead to massive bleeding and a fatal outcome^[40-42].

Moreover, central nervous system (CNS)-related symptoms may develop secondary to vascular events, such as sinus thrombus and intracranial aneurysms. Primary parenchymal involvement including meningitis and encephalitis, mostly in the pons and mesencephalon, is also seen in patients with BD. It is also known as Neuro-BD, accounting for 10% of patients. In addition, longitudinal extensive transverse myelitis (LETM), characterized by spinal cord lesions, may occur. Histopathological examination of neurological lesions typically shows an inflammatory cellular infiltration of the surrounding vessels^[40,41].

CD is a complex disorder, which primarily involves the small intestine and the colon. However, various extra-intestinal manifestations of the disease including oral and genital ulcers, erythema nodosum, uveitis, and arthritis may also be observed^[2]. Skin changes may be seen in 5%-10% of patients. Erythema nodosum (5.6%-13.5%), pyoderma gangrenosum (0.75%-0.15%), and acute neutrophilic dermatoses, also termed Sweet's disease, are among the main skin lesions. Other skin conditions include oral aphthous lesions, perianal lesions, large ulcers, fissures, fistulas, and aseptic abscesses^[47,48]. Pathergy positivity is extremely low in patients with CD, compared to those with BD^[49].

The most common ocular conditions are uveitis, episcleritis, conjunctivitis, and blepharitis. Non-granulomatous anterior uveitis may develop and recurrent episodes may result in permanent vision loss. Ocular complications are not associated with disease severity. Additionally, retinal vasculitis, which is extremely rare, has been reported in the literature as case studies^[47-51].

The clinical association between spondyloarthropathy and CD has been well-established. Nearly 10%-15% of CD patients are complicated by spondyloarthropathy. Both peripheral and axial arthropathies may be seen in CD. Peripheral arthropathies often present as asymmetric pauciarticular involvement. It is usually acute and self-limited, and the severity of the disease is reduced in parallel with decreased disease activity without sequelae. Persistent erosive monoarthritis has been described. Axial involvement resembles ankylosing spondylitis. Bilateral sacroiliitis, as well as spondylitis of the lumbar vertebrae and syndesmophytes may be seen. Chronic low back pain is the main symptom. It is frequent in asymptomatic sacroiliitis. Half of patients with CD have sacroiliac joint abnormalities, as evidenced by X-ray images^[52,53].

There are several studies showing a 1.5- to 3.5-fold increase in the risk of venous thromboembolism in CD. Some authors have suggested that it can be attributed to increased hospitalization and surgical interventions. On the other hand, the risk of arterial aneurysm and thromboembolism remain unchanged. However, mesenteric ischemia may occur^[54,55]. The incidence of Takayasu's

arteritis has been reported to increase in patients with CD^[56].

Primary sclerosing cholangitis is common in patients with CD, which has been reported in up to 10% of cases^[48]. Although neurological signs of CD are not evident, neuroradiological imaging studies have demonstrated alterations in brain morphology^[57].

Intestinal manifestations

Gastrointestinal manifestations are quite common in patients with BD. The most frequently observed signs include abdominal pain, diarrhea, nausea, anorexia, and abdominal distension. Despite the diffuse nature of the symptoms, ulcerations known as intestinal BD are relatively few. Gastrointestinal involvement varies regionally and according to the diagnostic method used. The incidence ranges from 15% to 50% based on symptoms alone and from 0.7% to 30% based on imaging or endoscopic findings^[58]. Gastrointestinal involvement is higher in patients with childhood-onset BD^[59]. BD-associated gastrointestinal involvement may affect all areas from the mouth to the anus. The terminal ileum and cecum are the main sites of ulcers, while few ulcers are seen in the esophagus and gastric duodenum. The most common site of involvement is in the segmental colon. Less than 15% of patients have diffuse intestinal involvement. The differentiation of intestinal BD from inflammatory bowel disease is sometimes quite challenging. The disease can be misdiagnosed as CD or ulcerative colitis during endoscopic examination. Fistulas, hemorrhage, and perforations mimicking CD may also be present. The shape of ulcers varies endoscopically from irregular to round and oval with a punched-out appearance, they are large (> 1 cm) and typically located in the deep layers. Longitudinal ulcers are rarely seen. The presence of less than six round and focal ulcers strongly indicate intestinal BD. Colonic ulcers include volcano-type and aphthous type lesions. Rectal and anal lesions are extremely rare^[36,60-62].

Abdominal pain, diarrhea with or without bleeding, fatigue, weight loss, and fever are common manifestations of CD. Odynophagia, dysphagia, and dyspeptic symptoms are also seen in the case of esophageal and gastroduodenal involvement. Diarrhea is a common presentation, but often fluctuates over a long period of time. Fibrotic strictures may lead to repeated episodes of small bowel, or less commonly colonic, obstruction. Transmural bowel inflammation is associated with the development of sinus tracts, which may give rise to a fistula or abscess formation. Perianal disease, such as anal fissures, perirectal abscesses, and anorectal fistulas, occur in more than one-third of patients with CD^[63]. CD may affect all areas from lips to the anus. Lesions were located in the terminal ileum in 40%-83%, colon in 32%, perianal region in 10%-15%, and the upper gastrointestinal tract in 4%^[2,58,63]. Endoscopic findings of proximal CD include mucosal edema, focal and diffuse erythema, nodular lesions, erosion, and ulcers^[64]. A diagnosis of CD should be considered in any patient who presents

with isolated terminal ileum involvement and ileoscopy should be performed in all patients. The earliest lesions in CD consist of tiny punched-out ulcers. Deeper ulcers can occur throughout the entire wall of the colon. Cobblestoning–Serpiginous and linear ulcers are seen along the longitudinal axis of the entire colon. CD lesions are discontinuous and can be adjacent to normal tissue. Rectal involvement is suggestive of ulcerative colitis rather than CD. In addition, perianal lesions are frequently seen in CD with fistula formation^[36,65].

PATHOLOGY

In BD, neutrophilic infiltration, lymphocyte aggregation of the surrounding vessels, and vascular proliferation have been observed in biopsy specimens of oral apthae and genital ulcers. Neutrophil-predominating infiltration, abscess formation and vasculitis-related changes may be present in skin lesions. Aggregation of lymphocytes, neutrophils, and eosinophils as well as edema and leukocytoclasia occur in the pathergy test site within the first 12 h. In the presence of large vessel involvement such as aortic involvement, medial elastic fiber ruptures or loss may be seen, while proliferation of the vaso vasorum and lymphocytic infiltration of the surrounding tissue may develop. Lymphocytic and necrotizing vasculitides are other conditions involving pulmonary arteries, veins, and septal capillaries. In addition, transmural necrosis and aneurysms of great vessels and pulmonary arteries may arise. Despite the non-specific nature, perivascular lymphocyte/plasma cell infiltration and myelin loss of parenchymal CNS lesions may develop^[26,27,66-68].

Furthermore, inflamed intestinal BD may lead to mesenteric vasculitis with ischemia or necrosis of the intestines. Ulcer specimens often show non-specific patterns, including fibrinopurulent exudates and necrotic debris in active ulcers and transmural fibrosis in chronic ulcers. Inflammation from the lumen to the serosa is present in the perforated site with mural necrosis. Vasculitic changes secondary to the inflamed surrounding tissue and thrombus formation in the small vessels including both arteries and veins are other critical manifestations. Lymphoid follicles may be seen due to mucosal erosion in some cases. The differential diagnosis of these lesions, which are histopathologically suggestive of CD is highly challenging^[26,68].

Histopathological characteristics of CD are discontinuous cryptic architectural abnormalities, mucin preservation at active sites, discontinuous inflammation, focal cryptitis, and epithelioid granulomas. Granulomas in histological sections are key features of CD, but are not necessary for diagnosis. In the submucosa, fibromuscular obliteration, nerve fiber hyperplasia and transmural lymphoid aggregates are found. Transmucosal increases in lamina propria cellularity and neutrophils are an indicator of disease activity^[69].

Both BD and CD may present with transmural enteritis and colitis. Longitudinal ulcers, cobblestone appear-

ance, and anorectal fistula are usual findings in Crohn's colitis. The presence of granulomas in biopsy specimens indicates CD, while vasculitides are suggestive of BD^[36].

DIAGNOSTIC CRITERIA

Although there is no specific diagnostic test for BD, diagnostic criteria sets described at different time points are available. The International Study Group (ISG) criteria^[70], which were defined in 1990, are the most commonly used criteria for the diagnosis of BD (Table 1). These criteria are based on the most frequent clinical signs of BD. In addition, some cases of CD meet these criteria^[71].

Several diagnostic and classification criteria for CD have been proposed^[8,72-75] (Table 1). The location and appearance of lesions are important for the diagnosis of CD. According to the Vienna^[74] and Montreal^[75] classifications, the diagnosis of CD is established by three variables: (1) age at diagnosis; (2) disease location; and (3) behavior of the disease. The Lennard-Jones criteria are based on endoscopic, surgical/histopathological, radiological and clinical findings^[73]. The Copenhagen criteria include histopathological confirmation of CD^[8]. A diagnostic criteria set for CD based on alterations in gastrointestinal morphology was published in 2011^[72]. However, no validated and widely adopted criteria set is currently available for the diagnosis of CD in clinical practice. The diagnosis usually relies on the patient history, physical examination, laboratory results, imaging studies, and endoscopic findings in combination with histopathological examination. Patients with BD, particularly with intestinal involvement, may be misdiagnosed and mismanaged as CD by clinicians with insufficient experience and knowledge on BD.

MANAGEMENT

As BD is a multisystem condition, effective management of the disease requires a multidisciplinary approach. Although the disease should be primarily managed by a rheumatologist, consultation is provided by a dermatologist, neurologist, gastroenterologist and cardiovascular surgeon, if necessary. The disease is inflammatory; therefore, immunosuppressive and immunomodulatory agents are first-line therapies. Due to the limited number of randomized-controlled clinical trials, management usually depends on the clinical experience of the treating physician. In 2008, the European League Against Rheumatism (EULAR) published a recommendation guideline for the management of BD^[76].

The management of patients with BD is based on the presence of organ involvement and disease severity. Colchicine is a widely used treatment for BD. Corticosteroids and azathioprine can be prescribed if colchicine monotherapy is inadequate. Colchicine is used for the management of mucocutaneous and musculoskeletal findings. Corticosteroids and azathioprine can be com-

Table 1 Diagnostic criteria for Behçet's disease and Crohn's disease

International Study Group Diagnostic Criteria for Behçet's disease ^[70]		Proposed diagnostic criteria for Crohn's disease		
		Japan Criteria ^[72]	Lennard-Jones Criteria ^[73]	Copenhagen Criteria ^[8]
Major findings	Recurrent oral ulcerations	A: Longitudinal ulcer B: Cobblestone-like appearance C: Noncaseating epithelioid cell granuloma	Typical diarrhea history for at least 2 mo; 1 Radiological features of diarrhea for more than 3 CD: segmental distribution, deep ulcerations or cobblestone pattern, thickened bowel wall, coarse mucosal relief, stenotic segments and fistulae;	1 History of abdominal pain, weight loss and/or 2 Characteristic endoscopic findings of ulceration (aphthous lesions, snail track ulceration) or cobblestoning or radiological features of stricture or cobblestoning
Minor findings	Recurrent genital ulcerations Eye lesions Skin lesions Positive pathergy test	(1) Irregular-shaped and/or quasi-circular ulcers or aphthous ulcerations found extensively in the gastrointestinal tract (2) Characteristic perianal lesions (3) Characteristic gastric and/or duodenal lesions	by endoscopy: patchy penetrating lesions, fisturing and strictures 3 Fistulas and/or abscesses with typical intestinal disease	3 Histopathology consistent with Crohn's disease (epithelioid granuloma of Langerhans type or transmural discontinuous focal or patchy inflammation) 4 Fistula and/or abscess in relation to affected bowel segments
Definite	Major finding plus two minor findings	1 Major finding A or B 2 Major finding C, with minor finding (1) or (2) 3 All minor findings (1), (2), and (3)	Positive findings or one positive plus the finding of granuloma	At least two of the criteria

bined in patients who are unresponsive to colchicine treatment and who have ocular, vascular, neurological, or intestinal involvement. Cyclosporine A and interferon- α are immunosuppressive agents used in the management of refractory uveitis and retinal vasculitis. A small number of patients with inadequate response may require mycophenolate mofetil and infliximab. Currently, these agents are used experimentally in the management of vascular involvement. In addition, cyclophosphamide is an effective immunosuppressive agent with increased side effects in patients with arterial, venous and neurological involvement who are refractory to other agents. Other agents that are preferred in unresponsive arthritis with a chronicity tendency include methotrexate and sulfasalazine. The latter is the most widely preferred agent in patients with intestinal BD, after corticosteroids and azathioprine. On the other hand, there are no randomized-controlled clinical trials in BD patients. Observational studies and case series have revealed that steroids, mesalazine, azathioprine, and sulfasalazine are likely to be used in the management of inflammatory bowel diseases. Recently, experience related to the use of anti-TNF agents have increased and some patients respond well to treatment. The efficacy of drugs in the treatment of CD and BD are compared in Table 2. In addition to immunosuppressive agents, antiaggregants, and anti-coagulants can be initiated in patients with venous and neurological involvement. However, no consensus on the use of antiaggregants and anti-coagulants has been reached yet, due to the low embolization tendency of BD-associated thrombosis and high bleeding

risk secondary to arterial aneurysms. In clinical practice, these agents are prescribed in patients with low bleeding risk^[7,41,76,77].

Corticosteroids have been used in the management of CD for over five decades. Corticosteroids are the most effective therapeutic agents in relieving disease exacerbations. They exert remarkable effects in suppressing pro-inflammatory cytokines and active lymphocytes and inhibiting inflammatory processes of the intestinal lamina propria. Although corticosteroids are more effective in higher concentrations, treatment-related side effects are likely to increase. Prednisolone treatment is usually initiated at 40-60 mg/d and reduced on a gradual basis. Nearly 48%-58% of the patients achieve complete remission, while 26%-32% achieve partial remission following 30 d of treatment. Approximately 16%-20% of patients are unresponsive. Six-mercaptopurine and its pro-drug azathioprine are the most commonly used agents in patients unresponsive to corticosteroids and maintenance therapy. Methotrexate is an alternative agent in patients who are intolerant or unresponsive to these agents. On the other hand, controversial data are available on the efficacy of 5-aminosalicylic acid (5-ASA) preparations. In several meta-analyses, mesalazine 4 g/d significantly reduced disease activity in patients with mild to moderate activity. All these agents are frequently prescribed due to their low side-effect potential^[78,79]. Anti-TNF agents including infliximab, adalimumab, and certolizumab pegol can be used in refractory patients with relapsing disease. Meta-analyses have demonstrated that anti-TNF agents are effective as both induction

Table 2 Treatment options for Behçet's and Crohn's disease

	BD		CD	
	Extraintestinal BD	Intestinal BD	Extraintestinal CD	Intestinal CD
Colchicine	S, M, A	-	-	-
Corticosteroids	All manifestations	+	All manifestations	+
Azathioprine	S, M, O, V, N	+	S	+
6-mercaptopurine	-	??	-	+
Cyclosporine A	O	-	-	-
Interferon-alpha	O, N	-	-	-
Mycophenolate Mofetil	O	-	-	-
Cyclophosphamide	O, V, N	-	-	-
Methotrexate	A, N	-	A, S	-
Sulfasalazine	A	+	A	+
Mesalazine	-	+	-	+
Anti-TNF agents	A, O, N	+	A, S, O	+

A: Arthritis; S: Skin; M: Mucosal; O: Ocular; V: Vascular; N: Neurological Involvement; (+): Effective; (-): Non-Effective; BD: Behçet's Disease; CD: Crohn's disease.

Table 3 Distribution of similarities and differences in the differential diagnosis of Behçet's disease and Crohn's disease [2,3,6,8,9,14,58,60,62,68,81]

	Behçet's Disease	Crohn's Disease
Gender (M/F)	4.9-0.57	2.9-0.76
Symptoms onset age (yr)	20.8-40	15-29
Average age at diagnosis (yr)	24.7-35.7	29.5-31
Oral aphthous ulcers (%)	Approximately 100	< 10
Uveitis (%)	57-69	< 10
Skin lesions (%)	61-87	< 10
Arthritis (%)	30-57	2-24.7
Gastrointestinal involvement (%)		
Ileocecal area	50-94	40-83
Colon	10-15	32-50
Upper GI	1-3	4
Perianal	1-2	10-15
Intestinal complications (%)		
Perforation	12.7	8.7
Fistula	7.6	24.7
Stricture	7.2	38.3
Abscess	3.3	19.6
Endoscopic Morphology	Round-oval shape, Focal, solitary Volcano-shaped Deep ulcers	Longitudinal ulcers with a cobblestone appearance (segmental and diffuse distribution)
Mucosal Biopsy	Vasculitis Neutrophilic infiltration Fibrinopurulent exudates Necrotic debris	Granuloma Focal cryptitis Nerve fiber hyperplasia Lymphoid aggregates

and maintenance therapy in CD patients with fistulizing disease^[80]. Surgery is indicated in patients with perianal involvement, fistulas, fissures, and intra-abdominal abscesses.

Medical and surgical management approaches for CD and intestinal BD are similar. Recently, a retrospective case series with long-term outcomes for both diseases was reported^[81]. Ten year-follow-up data after diagnosis showed no significant difference in the need for surgery between the study groups with CD and intestinal BD. However, CD patients required a higher dose of corticosteroids and immunosuppressive agents. The doses of biological agents were also higher in CD patients

compared to patients with intestinal BD (14.2% *vs* 1.4%). Based on these results, long-term prognosis appears to be similar in patients with CD and intestinal BD.

CONCLUSION

CD primarily involves the gastrointestinal system and can present with various extra-intestinal signs and symptoms. However, BD is a condition or syndrome that presents with multisystem involvement. The gastrointestinal tract is also one of the main sites of involvement in these patients. Both diseases have a true overlap, affecting the gastrointestinal tract. Furthermore, both

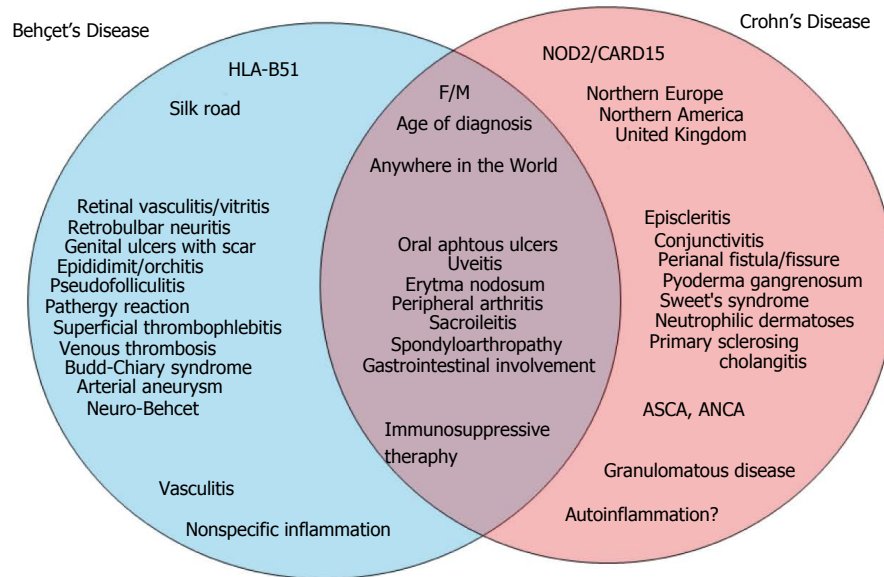


Figure 1 Similar and different characteristics of Behçet's disease and Crohn's disease. F: Female; M: Male; ASCA: Anti-Saccharomyces cerevisiae antibodies; ANCA: Anti-neutrophil cytoplasmic antibodies.

conditions share similar characteristics with respect to age of onset, gender, and inflammation biomarkers such as erythrocyte sedimentation rate and C-reactive protein (increased levels). Despite these similarities, the immunopathogenesis, genetic factors, and regional distribution are quite different. Although both diseases involve similar systems, they have distinct histopathological characteristics. For instance, uveitis is more common in BD, and CD patients are more likely to suffer from episcleritis or conjunctivitis. Figure 1 shows the similarities and differences in BD and CD. Table 3 summarizes the incidence of similarities, the distribution of gastrointestinal involvement, and endoscopic and histopathological differences.

As mentioned above, BD is more common in Asian and Mediterranean populations, while CD is more frequently seen in north European and American individuals. However, given the fact that we live in a globalizing world, the number of patients in whom the differential diagnosis of both conditions is of the utmost importance has increased. Therefore, rheumatologists and gastroenterologists who are mainly involved in the diagnosis and management of BD and CD should be well aware of the typical characteristics of both diseases.

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Multidisciplinary and evidence-based management of fistulizing perianal Crohn's disease

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improve outcomes.

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Core tip: This manuscript is a comprehensive review that focuses on the multidisciplinary management of fistulizing perianal Crohn's disease. The treatment options discussed in this review are based on a current literature review as well as our experience with the disease. Diagnostic and treatment algorithms are also provided.

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Abstract

Perianal symptoms are common in patients with Crohn's disease and cause considerable morbidity. The etiology of these symptoms include skin tags, ulcers, fissures, abscesses, fistulas or stenoses. Fistula is the most common perianal manifestation. Multiple treatment options exist although very few are evidence-based. The phases of treatment include: drainage of infection, assessment of Crohn's disease status and fistula tracts, medical therapy, and selective operative management. The impact of biological therapy on perianal Crohn's disease is uncertain given that outcomes are conflicting. Operative treatment to eradicate the fistula tract can be attempted once infection has resolved and Crohn's disease activity is controlled. The operative approach should be tailored according to the anatomy of the fistula tract. Definitive treatment is challenging with medical and operative treatment rarely leading to true healing with frequent complications and recurrence. Treatment success must be weighed against the risk of complications, specially anal sphincter injury. A full understanding of the etiology and all potential therapeutic options is critical for success. Multidisciplinary management of fistulizing perianal Crohn's disease is crucial to

INTRODUCTION

Although Gabriel^[1] first described patients with granulomatous perianal disease 17 years before the formal description of the disease by Burrill Crohn's^[2] in 1932, Bissell^[3] was the first to describe the associated perianal manifestations of Crohn's disease (CD). Furthermore, Morson *et al*^[4] documented the appearance of perianal non-caseating granulomas and fistulas many years before the onset of intestinal CD.

The reported prevalence of anorectal involvement in patients with CD has varied but the most current population-based series have found involvement in 14 to 38 percent of patients^[5-7], with isolated perianal disease seen in only five percent^[8]. The prevalence of perianal mani-

festations increases as the disease progresses distally, with up to 92 percent of patients with CD involving the colon and rectum developing fistulas^[9]. In most cases, bowel involvement precedes perianal disease^[9], but up to 40 percent of patients can experience perianal symptoms before intestinal manifestations^[10]. There does not seem to be a predilection for age but a younger age of onset increases the odds of developing perianal disease over time^[11-12].

The most common presentation of perianal CD is abscess and fistula. However, patients with CD are frequently affected by other perianal pathologies including hemorrhoids, fissures, skin tags, ulcers, and strictures. Perianal CD has been associated with a disabling natural history^[13], with common extraintestinal manifestations^[14] and greater steroid resistance^[15]. Perianal disease is often recurrent, with 35 to 59 percent of patients relapsing within two years^[16]. More than 80 percent of patients require operative treatment, and up to 20 percent may require proctectomy^[5,7]. Patients with perianal CD have also shown an increased risk for anal malignancies^[17,18], with active and long duration of disease being identified risk factors^[19-21].

The treatment of perianal CD continues to be a challenge, especially with the plethora of literature addressing both medical and operative treatment strategies. The purpose of this review is to summarize the efficacy of currently described methods for the management of fistulizing perianal CD and its complications.

ABSCESS

Abscesses usually occur with active perianal CD with an incidence of up to 62 percent during the course of the disease^[22]. Ischiorectal abscesses account for 40 percent of all perianal abscesses^[23]. Fistula tract location can influence abscess development and transsphincteric fistulas pose the greatest risk^[23].

Abscesses are uncommon with superficial fistula tracts. Makowiec *et al.*^[24] evaluated 61 patients with perianal CD and found that 73 percent of all abscesses were related to an ischiorectal fistula and 50 percent with a transsphincteric fistula. Recurrences occurred in 53 percent with a median time to recurrence of 14 mo. No patients with superficial fistula tracts had a second abscess, whereas about two thirds of patients with transsphincteric and ischiorectal fistulas recurred after 36 mo.

A detailed anorectal exam should be performed before any type of treatment is initiated. This frequently requires evaluation under anesthesia (EUA) with evaluation of the rectum to rule-out active disease. Perianal infection can occur in any anatomic plane (superficial, intersphincteric, ischiorectal, or supralelevator), and requires immediate drainage and treatment of systemic symptoms with broad-spectrum antibiotics^[6,25]. Many authors recommend drain placement or partial sphincter division to facilitate drainage, but these have not been associated with better outcomes^[26,27]. In the setting of

persistent perianal sepsis, imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT) are used to guide the drainage of deep or complex abscesses^[28,29].

When a fistula is encountered, a non-cutting seton should be placed to facilitate drainage and prevent recurrent infection, with improvement seen in 79 to 100 percent of patients^[30-35]. Long-term drainage with non-cutting setons without definitive therapy has been reported to result in fistula recurrence in 20 to 80 percent of cases^[33,36,37]. The combination of non-cutting setons and anti-tumor necrosis factor (TNF) therapy has been associated with fistula healing rates of up to 67 percent and will be discussed below^[38,39]. Fecal diversion to increase fistula healing and control perianal sepsis continues to be controversial with no level A data supporting its role but in the setting of persistent perianal sepsis, a temporary diverting stoma can be effective. Patients should be aware that these stomas are rarely reversed^[40].

Cryptoglandular abscess/fistulas can and do occur in patients with CD and should be recognized as so because treatment differs. These abscess/fistulas tend to be superficial and are not associated with active anorectal CD; therefore, anti-TNF therapy is not indicated. Abscess drainage should follow the same principles as mentioned above. Placement of a non-cutting seton is encouraged and any attempt of local surgical treatment should take into consideration the patients underlying continence and CD status. Supplemental imaging studies, such as endoanal ultrasound (EAUS), are very helpful even when cryptoglandular etiology is suspected.

FISTULA

A population-based study^[7] with up to 20 years of follow-up showed that one out every two patients with CD develop perianal fistulas. The etiology of perianal fistula formation in CD is not completely clear but genetic, microbiological, and immunological factors play a role. Most authors believe that fistulas originate either from the penetration of a rectal ulcer or from cryptitis spreading to the intersphincteric space. Intersphincteric and transsphincteric fistulas are the most common fistula tracts of cryptoglandular origin that occur in patients with CD. Suprasphincteric fistulas result from cryptoglandular disease or rectal ulceration, and extra sphincteric fistulas are frequently seen in patients with severe proctitis or iatrogenic injury.

At St. Marks Hospital, Tozer^[41] studied biopsy samples from Crohn's and idiopathic anal fistulas. Although immunological analysis showed no significant differences in interleukin (IL)-2, IL-4, IL-6, IL-10, TNF, and interferon levels, CD patients had significantly higher interleukin 17 levels and significantly lower CD65 levels. The authors showed data suggesting aberrant expression of homing molecules on dendritic cells in Crohn's anal fistulas suggesting a non-directed immune response, which may contribute to the pathophysiology.

Pelvic MRI is the preferred imaging study to assess fistulizing perianal disease. It has an accuracy of 90 percent for evaluating fistula tracts and of 97 percent for characterizing complex abscesses^[42,43]. Furthermore, operative management may be altered in ten to 20 percent of patients by the addition of MRI to EUA, and this increases to up to 40 percent in patients with CD^[44,45].

Once the anorectal disease is delineated, evaluation for proximal CD with endoscopy should also be considered. Although some studies have found an association between proximal fistulizing disease and perianal fistulas^[46], other investigators have not observed this finding^[47,48]. In patients with fistulizing perianal CD it is our practice to combine a pelvic MRI with EUA and rigid proctoscopy to evaluate for rectal inflammation.

Medical treatment

Once perianal infection is controlled, the fistula tract is characterized, and CD status is assessed; combined definitive medical and surgical therapy should be initiated (Figure 1). When active proctitis is encountered, this must be aggressively treated. Medical therapy includes antibiotics (metronidazole and ciprofloxacin), immunosuppressives (6-mercaptopurine, azathioprine, cyclosporine, and tacrolimus), and immunomodulators (infliximab, adalimumab, and certolizumab pegol). Although steroids are frequently used to manage concomitant luminal disease, there is no demonstrable role for corticosteroids in perianal CD. Medical treatment of perianal CD demands significant cooperation between gastroenterologists and surgeons as patient management is challenging and requires frequent feedback between medical professionals to optimize therapeutic strategies.

Antibiotic therapy

Antibiotics are commonly initiated when perianal infection is diagnosed and are frequently continued until immunosuppressive therapy is initiated^[49], with 70 to 95 percent of patients having a positive response within six weeks^[50,51]. It is our practice to continue antibiotic therapy for two weeks with perianal infection, and for three to four weeks with active proctitis. Metronidazole is the most common antibiotic prescribed for perianal CD and has been associated with fistula healing rates ranging from zero to 56 percent^[50,52,53]. Seventy-five percent of patients relapse after suspending treatment and side effects which include nausea and peripheral neuropathy commonly limit its long-term use.

Ciprofloxacin has been studied in small, uncontrolled series of patients with perianal CD^[54,55]. Improvement has been shown in approximately half of patients without detailed data on fistula healing. Ciprofloxacin was compared to metronidazole and placebo in a small randomized study including 25 patients^[53]. After receiving treatment for ten weeks, clinical remission and response were 30 percent and 40 percent with ciprofloxacin, 12.5 percent and 12.5 percent with placebo, and 0 percent and 14 percent with metronidazole; none of these dif-

ferences being significant.

Immunomodulators

The definitive medical treatment of perianal CD includes immunomodulation. A meta-analysis of five randomized controlled trials evaluated the efficacy of 6-mercaptopurine and azathioprine^[56]. Fistula healing occurred in 54 percent of patients *vs* 21 percent of controls (OR 3.09; 95%CI, 2.45 to 3.91). Intravenous cyclosporine has also shown to have a good response in up to 83 percent of patients^[57,58], but the effect is short-lasting when it is discontinued or transitioned to oral formulations^[59]. Tacrolimus has also been effective in the treatment of perianal CD, as shown in one randomized controlled trial. Clinical improvement was seen in 43 percent of patients *vs* 8 percent receiving placebo ($P = 0.004$)^[60].

Anti-TNF therapy

Anti-TNF therapy, which includes monoclonal antibodies that are given intravenously [Infliximab (chimeric – murine/human)] or subcutaneously [Adalimumab and Certolizumab pegol (human)], has shown good results in the multidisciplinary management of perianal CD. Most patients who receive anti-TNF therapy receive concomitant immunomodulators. This combination has been poorly studied, specifically in perianal CD, but may be associated with less perianal complications and increased fistula healing^[61]. What must be taken into consideration is that most studies evaluating anti-TNF therapy in the setting of perianal CD are of small numbers that involve heterogeneous patient groups with short follow-up. These studies also use varying definitions of fistula healing, disease improvement and “response”.

Infliximab alone: Present *et al*^[62] reported that three infusions of infliximab resulted in closure of perianal fistulas in 46 percent of patients over 3 mo follow-up. A large study from Hungary including 148 patients with CD reported a perianal fistula closure rate of 49 percent at a mean of 3 mo follow-up^[63]. A multicenter Italian study evaluating the impact of infliximab alone in 188 patients with perianal CD reported an initial response in 76 percent of patients with a 44 percent fistula closure rate^[64]. Ng *et al*^[65] prospectively evaluated the response to infliximab therapy with MRI in 34 patients with perianal Crohn's fistulas. At six months, 58 percent of patients showed fistula closure, with 37 percent showing good clinical response.

Infliximab plus surgery: Regueiro *et al*^[66] demonstrated an improved clinical response and less fistula recurrence when patients had EUA and seton placement before starting infliximab compared to patients who received infliximab alone. Topstad *et al*^[38] also achieved improved outcomes with combined seton drainage, infliximab infusion, and immunosuppressives in 29 patients. At a mean follow-up of nine months, 67 percent of patients showed a complete response. Hyder *et al*^[67] evaluated

long-term healing rates with this approach in 22 patients. At a median follow-up of 21 mo, the authors only observed an 18% fistula closure rate. Van der Hagen *et al*^[68] developed a multistep multidisciplinary approach that involved EUA with seton placement, fecal diversion when fistulas and abscesses recurred, infliximab therapy in case of persistent proctitis, and definitive fistula surgery. At a mean follow-up of 23 mo, fistula healing occurred in 90 percent of patients who received infliximab (9/10) compared to 71 percent in those who did not (5/7).

At the University of Minnesota, Gaertner *et al*^[39] evaluated the outcomes of 226 patients who underwent operative treatment for perianal Crohn's fistulas, with 79 of these patients also receiving preoperative infliximab. Fistula healing rates were similar regardless of infliximab therapy (59% *vs* 60%). However, patients who underwent surgery plus infliximab healed faster than those who did not receive infliximab (6.5 mo *vs* 12.1 mo; $P < 0.0001$), and seton placement plus infliximab infusion resulted in significantly improved fistula healing rates compared to seton placement alone ($P = 0.001$). Regardless of infliximab therapy, lay-open fistulotomy was the operation with the best healing rates. Active proctitis did not significantly impact healing after fistula surgery.

Adalimumab alone: Adalimumab has shown similar efficacy to infliximab in randomized controlled trials. In the CHARM (Crohn's trial of the fully Human Antibody Adalimumab for Remission Maintenance) study, 113 patients with perianal Crohn's fistulas received induction adalimumab; with subsequent maintenance adalimumab or placebo^[69]. Evaluation at 26 wk showed complete fistula closure in 30 percent of patients treated with adalimumab, with improved outcomes at 56 wk compared to placebo (33% *vs* 13%). The durability of these results have been confirmed at two years follow-up^[70]. In the CLASSIC-1 (Clinical Assessment of Adalimumab Safety and Efficacy Studied as an Induction Therapy in Crohn's disease) trial, adalimumab was compared to placebo with the aim to evaluate short-term outcomes^[71]. Thirty-two of 299 patients had perianal fistulas and no significant differences were observed in fistula healing.

Adalimumab has also been used in patients who have failed to respond to other anti-TNF agents, specially infliximab. In the GAIN (Gauging Adalimumab efficacy in Infliximab Nonresponders) trial, CD patients who were intolerant or who had lost response to infliximab received adalimumab or placebo^[72]. Forty-five of 325 patients had perianal fistulas and no significant differences in fistula healing were found between placebo and adalimumab. Based on these results, most physicians consider that a second biological agent has minimal efficacy in patients who have already failed anti-TNF therapy.

Adalimumab plus surgery: As the experience with anti-TNF therapy expands, many authors have reported on a combined approach with adalimumab and local anorectal procedures. Tozer *et al*^[73] reviewed the outcomes of 41 consecutive patients with fistulizing perianal CD

treated with infliximab ($n = 32$) or adalimumab ($n = 9$), and followed radiologically with MRI. Fifty-eight percent of all patients (66% infliximab and 43% adalimumab) demonstrated remission or response at three years. Fistula healing, as demonstrated by MRI, lagged behind clinical healing by a median of 12 mo. All patients who achieved radiological healing maintained fistula closure while on anti-TNF therapy but only 43 percent maintained fistula closure after cessation of anti-TNF agents. El-Gazzaz *et al*^[74] reviewed the Cleveland Clinic experience with combined anti-TNF therapy and anorectal surgery in 218 patients. Mean follow-up was 3.2 years. Two hundred and eighteen patients underwent operative treatment, 101 with anti-TNF therapy (74 infliximab and 27 adalimumab). Patient groups were comparable in demographic data and CD history but operative treatment was significantly heterogeneous. Patients who received combined anti-TNF therapy and surgery had significant overall improvement compared to patients who underwent surgery alone (36% *vs* 71%, $P = 0.001$).

Local anti-TNF therapy: Local injections of anti-TNF agents have also been attempted in fistulizing perianal CD, specifically in patients with contraindications to systemic treatment and resistance to infliximab. Poggioli *et al*^[75] performed three to 12 local injections of infliximab (15-20 mg) adjacent to both internal and external openings and fistulous tract in 15 patients. Fistula closure occurred in ten patients at a mean follow-up of 18 mo. Asteria *et al*^[76] achieved clinical response in six of eleven patients treated with local infliximab. Four of the eleven remained healed at a median of ten months of follow-up.

Tonelli *et al*^[77] reviewed the outcomes of 12 patients with fistulizing perianal CD who underwent local injection of Adalimumab. Each patient received a median of seven (range, 4-16) injections. At a mean follow-up of 17.5 mo, 75 percent of patients (9 of 12) no longer had fistula drainage, and three patients (25%) showed clinical improvement. No adverse side effects were noted.

Certolizumab pegol: Certolizumab pegol is a humanized monoclonal antibody directed against TNF alpha. The antibody is fused with polyethylene glycol, which does not cross the placenta, so it should be safe in pregnancy. In 2008, the Food and Drug Administration approved Certolizumab pegol for the treatment of CD. Schreiber *et al*^[78] evaluated its impact in patients with fistulizing CD. Patients with fistulizing CD from a randomized controlled study (PRECISE 2, $n = 108$) comparing certolizumab pegol *vs* placebo were further randomized (if a good initial response was noted) to certolizumab pegol ($n = 28$) or placebo ($n = 30$) every four weeks until 24 wk. The majority of patients (55/58) had perianal fistulas. At week 26, fistula closure occurred in 36 percent of patients in the certolizumab pegol group compared to 17 percent of patients receiving placebo ($P = 0.038$).

Operative treatment

If the attempt to heal a fistula has significant impact on

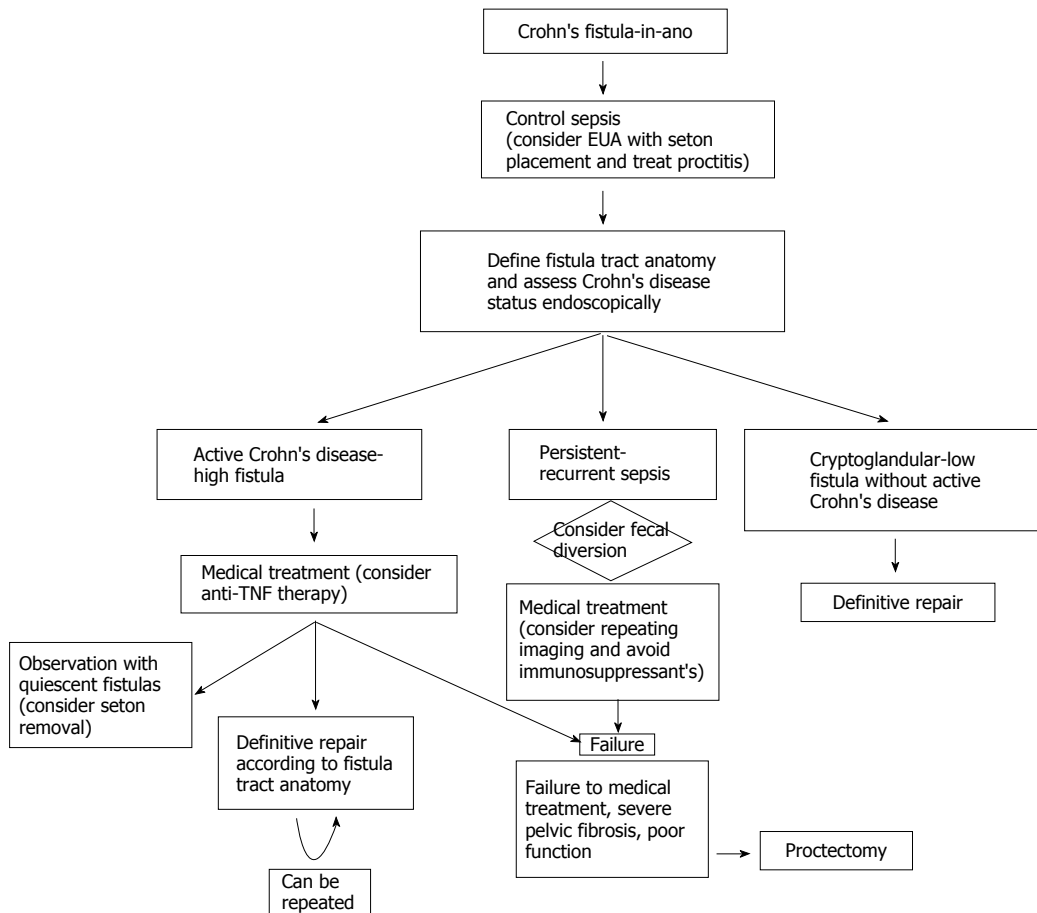


Figure 1 Diagnostic and treatment algorithm for fistulizing perianal Crohn's disease.

a patient's quality of life, operative treatment should be undertaken. Currently, the majority of operations for fistulizing perianal CD are performed in conjunction with medical therapy (immunomodulators or anti-TNF agents), and because this approach has been covered above, this section will focus on operative indications and efficacy of the most popular surgical techniques.

Most low, simple fistulas can be treated by fistulotomy. Healing rates from 80 to 100 percent have been reported with this technique^[27,31,79,80]. Despite careful patient selection, an occasional fistulotomy wound may result in a chronic ulcer. In this situation, medical treatment is preferred as further operations have been associated with recurrent infection, fistula, and sphincter damage.

If partial sphincter division would compromise fecal continence, one can choose between minimally invasive techniques and anorectal repairs. Minimally invasive techniques include fibrin glue injection and collagen plug insertion. These techniques have no significant effect on fecal continence, are well tolerated by the patient, can be repeated, and are associated with fistula healing rates between 38 and 71 percent^[81-84]. Fistula recurrence is common and occurs in approximately 50 to 70 percent of patients^[81-84]. Video-assisted anal fistula treatment (VAAFT) and local injection of adipose-derived stem

cells are recently described minimally invasive techniques that have been employed in patients with fistulizing perianal CD^[85,86]. VAAFT involves performing fistuloscopy to identify the etiologic crypt and rule-out secondary tracts and then excise the internal opening. After this, the fistula tract is fulgurated. Stem cell therapy is a novel and promising approach for the treatment of chronic inflammatory conditions, and its use in fistulizing perianal CD has increased in Europe. Fistula healing rates between 30 and 82 percent have been reported with these techniques but the long-term safety and outcomes have not been adequately studied in the Crohn's population. Overall, studies assessing the efficacy of minimally invasive techniques for Crohn's perianal fistulas tend to be of small patient numbers, non-comparative and heterogeneous patient groups, retrospective nature, and with short duration of follow-up.

The most commonly employed anorectal operation for transsphincteric Crohn's fistulas is a rectal advancement flap. This procedure has been associated with incontinence rates between five and nine percent but has not been associated with an increased risk for proctectomy^[87]. Contraindications include significant proctitis, a cavitating ulcer or anal stenosis. Crohn's fistula healing rates reported in the literature average 64 percent^[87]. A recently described technique, ligation of intersphinctic-

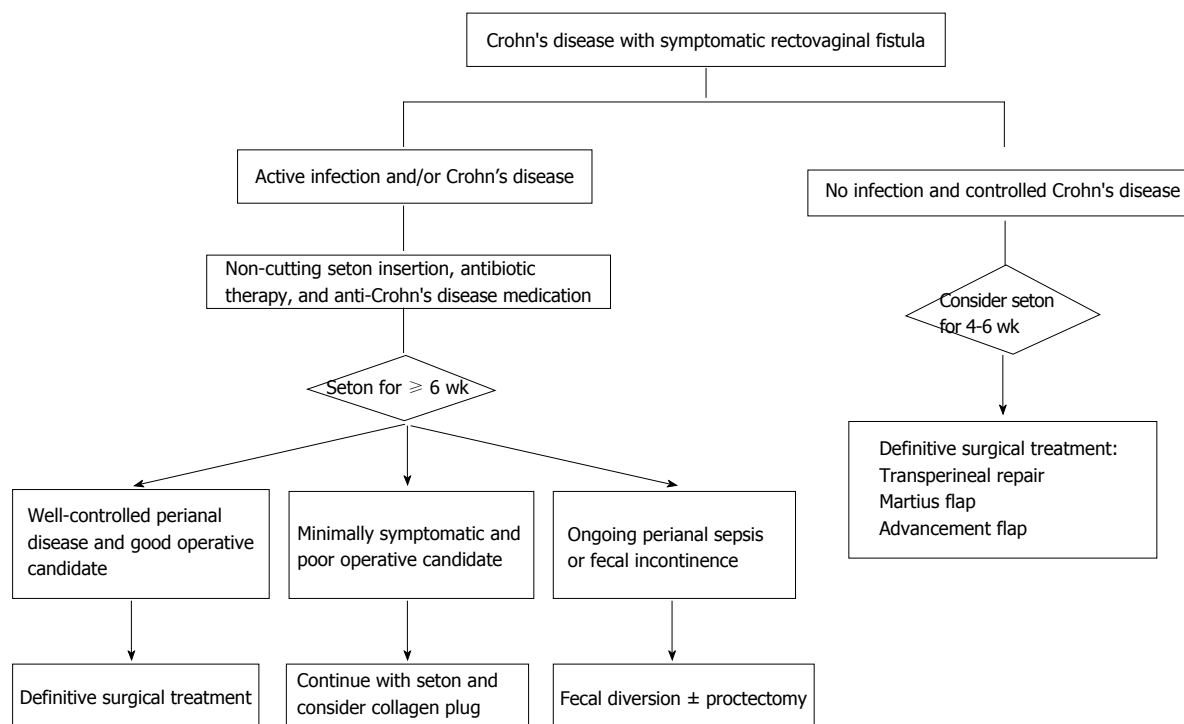


Figure 2 Treatment algorithm for patients with Crohn's disease and symptomatic recto-vaginal fistulae.

teric fistula tract (LIFT) which involves the identification and ligation/transection of the fistula tract in the intersphincteric plane, is being increasingly employed in patients with transsphincteric Crohn's fistulas^[88]. This technique also has minimal to no repercussion on fecal continence but does involve a perianal wound. Although encouraging results have been reported in complex fistulas of cryptoglandular origin, experience in CD patients is limited^[89,90].

In the setting of a large anal canal ulcer or severe stricture, an endorectal advancement flap can be performed in selective patients^[91]. After the ulcer or stricture is excised, a full-thickness circumferential sleeve is mobilized and a formal rectoanal anastomosis is performed in combination with a diverting loop ileostomy.

SPECIFIC SITUATIONS

Rectovaginal fistulas

After obstetric trauma, CD is the second most common cause of rectovaginal fistula (RVF)^[92], occurring in five to 23 percent of CD patients^[93-95]. The majority of RVF's in the setting of CD are low and transsphincteric, and arise from rectal ulceration or infection of anterior anal glands^[94,96].

The management of RVF in CD is challenging. Treatment depends on the degree of symptoms, CD activity, and the anatomy of the fistulous tract (Figure 2). Minimally symptomatic patients may not require any treatment^[7,94,97,98]. However, carefully selected symptomatic patients should be treated with a step-wise multidisciplinary approach. Drainage of local infection, seton

placement and medical therapy are the initial steps before any attempts at fistula closure^[92,94].

Patient selection is very important. Women with extensive anorectal CD are not good candidates for definitive fistula operations without first eradicating local infection and controlling the activity of underlying CD. Contrast to what has been reported in non-CD patients, a previous failed repair does not dictate a worse outcome with a subsequent operation. Healing rates reported after secondary operations are similar to those seen after a first attempt repair (29%-54%)^[99-101]. Fecal diversion to protect a repair is also controversial. Penninckx *et al*^[99] evaluated the impact of fecal diversion and parenteral nutrition in 32 consecutive patients undergoing RVF repair and did not find any significant role for either of these interventions. However, when O'Leary *et al*^[102] selectively used fecal diversion in a step-wise approach that included initial seton placement and delayed repair, fistula healing occurred in 80 percent of patients. A diverting stoma does not ensure fistula healing and should only be performed in complex and recurrent cases.

Most of current treatment algorithms include combined medical and operative treatment. Present *et al*^[103] found that 6-mercaptopurine was more effective than placebo, when combined with surgery (31% *vs* 6%). Most RVF's recurred after discontinuation of 6-mercaptopurine. Similar results were observed with cyclosporine in two studies that included a total of six patients with RVF^[104,105].

El-Gazzaz *et al*^[106] evaluated long-term outcomes in 65 women with Crohn's RVF's who underwent a variety of different procedures. At a median follow-up of

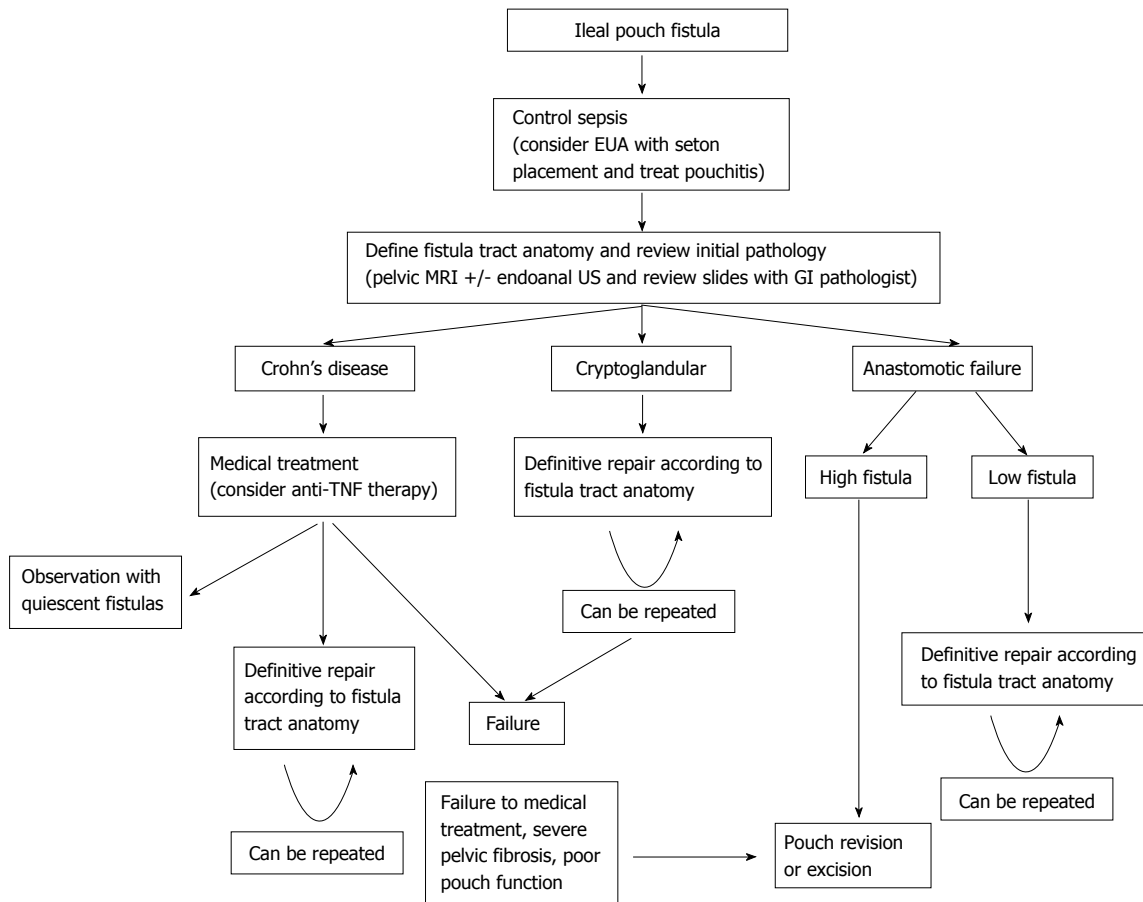


Figure 3 Diagnostic and treatment algorithm for patients with ileal pouch fistulas.

47 mo, 46 percent healed. Multivariate analysis showed that immunomodulators were associated with successful healing ($P = 0.009$); and smoking and steroids were associated with failure ($P = 0.04$).

The efficacy of infliximab in RVF and CD has been controversial^[38,62,66-68,107-109]. In the ACCENT II study^[109], the initial response rate to infliximab was 64 percent. Rectovaginal fistula closure was maintained for longer with maintenance infliximab compared to placebo (46 wk *vs* 33 wk). Gaertner *et al*^[110] reviewed the outcomes of 51 patients with Crohn's RVF's who underwent combined medical and operative treatment, 26 received preoperative infliximab. At a mean follow up of 38.6 mo, 27 fistulas (53%) healed. Transperineal repair was the operation with the highest healing rate regardless of infliximab therapy. Preoperative fecal diversion, active proctitis and infliximab therapy did not significantly impact fistula healing.

The definition of fistula healing tends to raise controversy when reviewing the RVF literature and seems to be influenced by the type of treatment, method of evaluation, and follow-up period. Rasul *et al*^[111] assessed RVF healing by endoanal ultrasound in patients who clinically healed with infliximab therapy. Only five of 35 women demonstrated improvement but none showed fistula closure on ultrasound. Bell *et al*^[112] found good correlation between clinical assessment and MRI in seven of ten patients treated with

infliximab. Only two of these patients had RVF.

Ileal pouch fistulas

Patients who develop CD after restorative proctocolectomy with ileoanal anastomosis are at particularly high risk of developing pouch-anal fistulas. Although pre-operative colorectal pathology, operative technique, and postoperative pelvic sepsis have also been identified as risk factors, CD is considered the most common^[113-115]. Several operative techniques have been described to control pelvic and perianal sepsis and ultimately eliminate the fistula tract^[116-120], but because of the low incidence of these fistulas, the optimal management continues to be controversial (Figure 3). Gaertner *et al*^[121] reviewed the outcomes of 25 patients who presented with symptomatic ileal pouch fistulas over a 22-year period. Fistulas were classified as pouch-anal (48%), pouch-vaginal (28%), complex (16%), and pouch-perineal (8%). The most common etiology was CD. Overall fistula closure with a variety of local anorectal and abdominal procedures was 64 percent at a median follow-up of 29 mo. Postoperative pelvic sepsis, fecal diversion, and anti-TNF therapy did not significantly impact fistula healing. Three patients (12%) required pouch excision with end ileostomy.

Fistula-associated cancer

In 1934, Rosser^[122] first described carcinoma associated

with a chronic perianal fistula. Fistula-associated adenocarcinoma is a rare but increasingly reported malignancy^[18,21,123-131] that is commonly found in CD patients with chronic anal fistulas^[18,21]. This malignancy is frequently associated with chronic, complex fistulas and can be particularly difficult to diagnose. High clinical suspicion is crucial to avoid any delay in diagnosis and treatment. Chronic infection and inflammation (*i.e.*, CD and radiation) are the most frequently associated risk factors but even when the diagnosis is suspected clinically, confirmation requires EUA with biopsy. Misdiagnosis commonly occurs in elderly patients and patients with long-standing anorectal disease. Once the diagnosis of cancer has been established, EAUS and MRI are recommended for staging^[132].

Mucinous adenocarcinoma is the most common malignancy reported in long-standing perianal fistulas. It is typically a slow growing, locally aggressive neoplasm that mainly spreads *via* the inguinal lymphatic's^[133]. Outcomes are good when malignancy is diagnosed early^[131,133-136]. Oncologic resection remains the standard treatment option. Abdominoperineal resection is the most frequently employed operation^[131,137,138]. The role of neoadjuvant chemoradiotherapy in the treatment of this neoplasm has not been well studied, probably because of its rarity, but results are promising^[21,131]. Neoadjuvant therapy may play a significant role to improve outcomes but remains investigational.

Gaertner *et al.*^[131] identified 14 patients with fistula-associated anal adenocarcinoma. The most common presentation was persistent perianal fistula ($n = 9$). Ten patients (71%) had CD. Abdominoperineal resection was performed in eleven patients, seven following neoadjuvant chemoradiotherapy. At a mean follow-up of 64 mo, ten patients were alive without evidence of disease and four patients died with metastatic disease. All seven patients who received neoadjuvant chemoradiotherapy had a complete pathologic response. In a systematic review by Iesalnieks *et al.*^[21], a total of 23 publications including 65 patients with fistula-associated adenocarcinoma and CD were reviewed. Abdominoperineal resection was performed in 56 patients with an overall 3-year survival rate of 54 percent.

We recommend that tissue from refractory, recurrent and chronic anal fistula tracts, regardless of their etiology, should be submitted for pathologic evaluation. All patients with long-standing perianal CD should undergo cancer surveillance. Although the impact of neoadjuvant chemoradiotherapy remains controversial, oncologic resection continues to be the standard treatment option for fistula-associated adenocarcinoma.

Proctectomy

Proctectomy is appropriate in patients in whom repeated medical and operative strategies fail. Historically, it is required in ten to 20 percent of patients with perianal CD^[6], and is commonly associated with perineal wound breakdown, chronic open wounds and sinus formation

in up to half of patients^[139,140]. In our experience, intersphincteric proctectomy (when feasible) and the use of rectus abdominal and gracilis flaps can help with avoiding these complications.

A low Hartmann's procedure is an alternative approach that may result in a healed perineum in up to 60 percent of patients with perianal CD^[141]. Despite this approach, Guillem *et al.*^[142] reported a 54 percent completion proctectomy rate in 28 patients who underwent rectal exclusion, plus an additional nine patients had persistent active disease at the rectal stump.

CONCLUSION

The appropriate treatment of fistulizing perianal CD must be individualized to each patient. The primary goals are to ameliorate symptoms and prevent complications. Overall, a less aggressive approach is preferred as many patients will require repetitive operations that can often result in outcomes that are worse than the disease itself.

Based on the current literature, multidisciplinary treatment includes: eradication of infection, assessment of CD status and fistula tract(s), medical therapy, and selective operative management. The first phase of treatment is to drain the perianal infection. This typically involves an EUA, seton drainage and a short course of antibiotics. The second phase consists of endoscopically evaluating the extent of CD and delimiting the anatomy of the fistula tract with EUA and either EAUS or MRI, or both. During this phase, medical therapy with immunomodulators and anti-TNF agents is typically initiated but if the fistula is thought to be of cryptoglandular etiology, CD medications are rarely required.

The third phase should ideally involve healing of the perianal pathology. Many patients who have minimal symptoms elect to continue with a non-cutting seton or removal and expect healing in some cases. On many occasions a non-cutting seton may actually act as a cutting seton, specially in low superficial fistula tracts. The extensive range of operations highlights the complexity of operative treatment. These include a variety of minimally invasive techniques and anorectal operations. Sphincter injury and fecal incontinence should be the main concern with any anorectal operation. The operative approach depends on the anatomy of the fistula tract, CD status, and the patients' functional status. Attempts to heal a fistula in the setting of active infection and proctitis are likely to fail. If the patient's symptoms persist or increase despite adequate medical and surgical treatment, a diverting stoma or proctectomy should be considered.

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Predictors of response to anti-tumor necrosis factor therapy in ulcerative colitis

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may predict response to anti-TNF drugs in UC would help to better select for patients with a high probability to respond and minimize risk and costs for those who will not respond. Analysis of the major clinical trials and the accumulated experience with the use of anti-TNF drugs in UC has resulted to the report of such prognostic factors. Included are clinical and epidemiological characteristics, laboratory markers, endoscopic indicators and molecular (immunological/genetic) signatures. Such predictive parameters of long-term outcomes may either be present at the commencement of treatment or determined during the early period of therapy. Validation of these prognostic markers in large cohorts of patients with variable characteristics will facilitate their introduction into clinical practice and the best selection of UC patients who will benefit from anti-TNF therapy.

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Key words: Ulcerative colitis; Infliximab; Adalimumab; Anti-tumor necrosis factor; Predictors of response; Personalized treatment

Abstract

Ulcerative colitis (UC) is an immune-mediated, chronic inflammatory disease of the large intestine. Its course is characterized by flares of acute inflammation and periods of low-grade chronic inflammatory activity or remission. Monoclonal antibodies against tumor necrosis factor (anti-TNF) are part of the therapeutic armamentarium and are used in cases of moderate to severe UC that is refractory to conventional treatment with corticosteroids and/or immunosuppressants. Therapeutic response to these agents is not uniform and a large percentage of patients either fail to improve (primary non-response) or lose response after a period of improvement (secondary non-response/loss of response). In addition, the use of anti-TNF agents has been related to uncommon but potentially serious adverse effects that preclude their administration or lead to their discontinuation. Finally, use of these medications is associated with a considerable cost for the health system. The identification of parameters that

Core tip: The use of anti-tumor necrosis factor (TNF) monoclonal antibodies for the treatment of ulcerative colitis has been associated with high rates of primary and secondary non-response, important safety issues and considerable cost. Selection of patients with the highest probability to response to anti-TNF treatment would overcome these problems. Analysis of the pivotal trials and accumulated experience from clinical practice has led to the identification of certain prognostic factors for favorable or adverse outcomes. These include clinical and epidemiological parameters, biological markers of inflammation, endoscopic findings, molecular signatures and pharmacological factors. Incorporation of such predictors into the current therapeutic protocols may lead to the optimization of anti-TNF treatment in ulcerative colitis.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon, which affects almost 0.1% of the Western population^[1]. Its natural history is dominated by chronic, relapsing intestinal inflammation, extra-intestinal involvement, and the development of long-term complications, which lead a considerable percentage of patients to colectomy.

Treatment for UC has been traditionally aimed against controlling acute and chronic inflammation^[2]. Conventional therapy consists of 5-aminosalicylic acid (5-ASA) compounds, whereas more severe cases are handled with steroids during the acute phase and immunosuppressants (thiopurines) as the maintenance regimen. Despite the proven efficacy of these drugs, a significant number of patients do not accomplish durable remission and/or experience side effects. Furthermore, there has been a change in the therapeutic goals in UC in recent years. Traditionally, such goals have been considered the achievement of clinical remission and the avoidance of colectomy. Nowadays, however, it has become clear that treatment should include the complete elimination of active inflammation in the colon without long-term use of corticosteroids. In this context, mucosal healing and deep remission which both indicate the absence of endoscopically and biologically (*i.e.*, serological and/or fecal inflammatory markers) evident inflammation may be the ultimate endpoint. The accomplishment of such demanding endpoints has been linked to better long-term outcomes including colectomy and cancer prevention^[3].

In recent years, treatment of UC has been revolutionized by the therapeutic application of monoclonal antibodies against tumor necrosis factor (TNF) as these agents offer effective long-term treatment for the most difficult cases.

ANTI-TNF TREATMENT IN UC

There are currently three monoclonal antibodies against human TNF that are licensed for the treatment of UC, infliximab (IFX), adalimumab (ADA), and Golimumab^[4]. The data regarding Golimumab are limited. Therefore, our review will focus on IFX and ADA. IFX is a chimeric mouse/human IgG1 antibody that is administered intravenously. On the other hand, ADA is a humanized IgG1 antibody administered as subcutaneous injection. The two clinical scenarios for anti-TNF therapy in UC are: firstly, outpatient cases with moderate to severe UC who are refractory or intolerant to first-line treatment; and, secondly, patients with acute severe disease refractory to intravenous steroids^[4]. In regards to the latter

scenario, data exist only for IFX.

Recent clinical trials have established the efficacy of anti-TNF treatment in UC. In the two pivotal IFX trials, ACT 1/2, the primary short-term (8 wk) response of moderate to severe UC to IFX has been reported to be 65.5%/69.4% for clinical response and 33.9%/39% for remission, respectively (dose regimen 5 mg/kg at 0-2-6)^[5]. Among patients who responded to the induction regimen nearly 50% maintained their response at week 30. Similarly, in the definitive clinical trial (ULTRA) for ADA, short-term response at week 8 was achieved in nearly 50% of patients, whereas long-term remission rate at week 52 was 17%^[6].

Despite these encouraging results, the use of anti-TNF monoclonal antibodies is compromised in clinical practice by certain issues of efficacy and safety. Anti-TNF failure is an intriguing issue as it may be attributed to both disease characteristics and the drugs' interference with the immune system. Primary non-response is characterized by lack of response to induction therapy. The incidence ranges between 20%-40% for both anti-TNF agents. Switching to another drug is common practice, with a success rate of more than 50%^[7,8]. On the other hand, loss of response is defined as the recurrence of the patient's symptoms following successful induction of remission. In the case of CD it has been estimated between 23%-46%^[9], whereas no solid data exists for UC. It is believed that immunogenicity underlies secondary failure, as antibodies against anti-TNF drugs and reduced trough levels have been implicated in the majority of studies^[10-12]. Optimization of treatment (dose increase and/or shortening of the administration interval) leads to recovery of response in 60%-90% of patients^[10].

The use of anti-TNF has also been associated with safety concerns. Among the most fearful ones are: severe infectious including reactivation of latent tuberculosis, neurological manifestations and risk of neoplasia. In addition, infusion reactions and delayed hypersensitivity to IFX occurred in 10% and 1% of patients, respectively, in the ACT trials. The most significant side effects are probably associated with long-term administration and combination with other immunomodulatory medications. It should be noted that in the ACT and ULTRA studies there were no differences between active drug and placebo.

Taken together, it is currently evident that fine-tuning of the use of anti-TNF therapy in UC is required. The ultimate goal should be to achieve maximum efficacy with a minimum risk for side effects. When therapeutic strategies are designed the following parameters should be taken into consideration: (1) the patients who receive anti-TNF therapy are the ones with the most difficult-to-treat disease; (2) the drugs' efficiencies are far from perfect with high rates of primary and secondary failures; (3) the potential for serious side effects especially with chronic use; and (4) the high cost of these medications. One significant way to address these problems and optimize the clinical use of anti-TNF agents would be

Table 1 Prognostic indicators of response to anti-tumor necrosis factor treatment in ulcerative colitis

At initiation of treatment	During treatment
Clinical and epidemiological parameters	
Severity of the disease	Early clinical response
Younger age	
Duration of colitis < 3 yr	
Extensive colitis	
Laboratory indicators	
CRP	Low CRP at week 12
Hemoglobin	Drop of serum CRP
Serum albumin	Fecal calprotectin
Immunological and genetic markers	
p-ANCA	Gene expression profiling
Pre-treatment mucosal TNF- α expression	Percentages of regulatory T cells
Mucosal expression of IL-17 and IFN- γ	
Genetic polymorphisms	
Endoscopic findings	
	Mucosal healing
Treatment-related factors	
Pharmacological history	Number of IFX infusions
Exposure to immunosuppressants	Co-administration of immunosuppressants
Response to prior treatment with infliximab	Escalation of anti-TNF therapy
	IFX trough levels
	Antibodies against anti-TNF

CRP: C-reactive protein; p-ANCA: Perinuclear antineutrophil cytoplasmic antibodies; TNF: Tumor necrosis factor; IL: Interleukin; INF: Interferon; IFX: Infliximab.

to carefully select patients in whom there is decreased probability for primary or secondary non-response. Such an approach will ensure that the patients who receive the medications are those who will most probably benefit. As almost ten years have passed since the initial application of anti-TNF therapies in UC, analyses of the pivotal clinical trials and accumulation of clinical experience has allowed the identification of such factors that signify a better response to these treatments (Tables 1 and 2). It is the purpose of the current review to summarize information regarding prognostic markers for response to anti-TNF monoclonal antibodies in patients with UC.

PREDICTORS OF RESPONSE

Prognostic factors at the initiation of anti-TNF treatment

Clinical and epidemiological parameters: Several studies have looked into the effect that the severity of the UC episode may have on the response to anti-TNF administration. In a study by Jürgens *et al*^[13], 90 UC outpatients were treated with IFX and followed for 14 wk. Disease activity was quantified by use of the Colitis Activity Index (CAI). Nearly half of the patients achieved early remission at week 14. Overall, the mean CAI dropped from 10.4 points at baseline to 5.0 at week 14 ($P < 0.001$). The authors reported a significant positive association between UC activity and response to treatment with IFX. It should be noted, however, that only a small number of severe cases were included in this study.

In a second report, 191 UC patients who received at least one infusion of IFX between 2000 and 2009 were analyzed with the aim to identify predictors of response^[14]. Mean follow-up was 18 mo. Failure outcomes

included primary-non response, dose-escalation, colectomy and hospitalization, which were noted in 22%, 45%, 19% and 36% of patients, respectively. In contrast to the study by Jürgens, administration of IFX for the indication of acute severe colitis was associated with a 3-fold risk for unfavorable outcome.

Park *et al*^[15] studied 89 Korean patients with moderate to severe UC who were treated with IFX. Following induction, 59 patients exhibited clinical response at week 8 (66.3%). None had a colectomy within one year, in contrast to 11/30 of those who did not respond. Predictors of primary non-response to the drug were the severity of the disease before initiation as well as prior cytomegalovirus (CMV) infection of the colon. Patients with a pre-treatment Mayo score ≥ 11 had an increased risk of colectomy (OR = 5.05, $P = 0.007$).

Analysis of the large clinical trials ACT 1 and 2- offers additional information regarding prognostic factors for colectomy (*i.e.*, failure of IFX) in patients with moderate to severe UC^[16]. As reported by Sandborn *et al*^[16], 630 patients who participated in the ACT trials had a complete follow-up for colectomy. A baseline Mayo score of ≥ 10 strongly increased the risk for colectomy (HR = 1.84, $P = 0.01$).

Prognostic indicators for response to ADA in UC have also been reported recently. A placebo controlled trial of ADA for UC patients with refractory disease who were naïve to biologics evaluated the short-term efficacy of the drug^[17]. At week 8, 18.5% were in remission ($P = 0.031$ *vs* placebo). Study analysis identified a trend towards less efficacy in cases of more severe disease at baseline. Patients with Mayo score ≥ 10 , CRP ≥ 10 mg/L and extensive disease responded less favorably

Table 2 Clinical trials that reported prognostic indicators for response to anti-tumor necrosis factor treatment in Ulcerative Colitis

Ref.	Type of study	No. of patients	Anti-TNF drug	Response endpoints	Predictor of response
Arijs <i>et al</i> ^[26]	Cohort		IFX	Endoscopic and histological healing	Mucosal gene expression signature
Armuzzi <i>et al</i> ^[31]	Retrospective	88 (78.4% IFX experienced)	ADA	Clinical remission (4-54 wk)	Short-term clinical remission Low CRP at week 12 (remission at week 54) ¹ Previous immunosuppressant use (lower long-term remission rates)
Armuzzi <i>et al</i> ^[27]	Prospective	126	IFX	Steroid-free clinical remission Mucosal healing Colectomy (12 mo)	Thiopurine-naïve status Combination treatment CRP drop to normal
Ben-Horin <i>et al</i> ^[10]	Retrospective	62 (CD/UC)	IFX	Loss or response	¹ Low trough levels Anti-infliximab antibodies
Cesarini <i>et al</i> ^[39]	Retrospective	41 (secondary loss of response)	IFX	Clinical remission Colectomy-free (52 wk)	Rapid clinical response to optimization
Colombel <i>et al</i> ^[3]	Prospective (ACT trials)	728	IFX	Clinical remission Clinical response Colectomy	Mucosal healing at week 8 (predictive of long-term outcome)
De Vos <i>et al</i> ^[32]	Prospective	53	IFX	Mayo clinical score Endoscopic remission	Fecal Calprotectin
Fasanmade <i>et al</i> ^[23]	Retrospective	728	IFX	Trough levels Clinical response	¹ Serum albumin concentration
Ferrante <i>et al</i> ^[21]	Cohort	121	IFX	Colectomy-free survival (33 mo)	Short term clinical response CRP > 5 mg/L ¹ Previous iv treatment with steroids/cyclosporin
Ferrante <i>et al</i> ^[18]	Cohort	100	IFX	Early clinical response	Younger age pANCA-/ACSA+
Garcia-Bosch <i>et al</i> ^[28]	Retrospective	48	ADA	Clinical response (partial Mayo score) Colectomy (week 54)	Response to prior treatment with infliximab Early response to adalimumab
Gonzalez-Lama <i>et al</i> ^[20]	Retrospective	47	IFX	Clinical response Steroid-free remission Colectomy	¹ Disease extent
Gustavsson <i>et al</i> ^[35]	Placebo controlled trial	45	IFX	Colectomy (3 yr f-up)	Mucosal healing at 3 mo
Jakovovits <i>et al</i> ^[19]	Retrospective	30	IFX (not standard induction regimen 0-2-6)	Colectomy	¹ Younger age at diagnosis
Jürgens <i>et al</i> ^[13]	Retrospective	90	IFX	Clinical response Clinical remission (week 14)	CAI-disease activity ANCA seronegativity IL23R genotype
Lee <i>et al</i> ^[22]	Retrospective	134	IFX	Clinical response Clinical remission	Haemoglobin > 11.5 CRP > 3 Immunomodulator-naïve status Response at week 2 Mucosal healing
Kohn <i>et al</i> ^[36]	Open label	83 severe colitis	IFX	Colectomy/Death > 2 mo after first infusion (median f-up 23 mo)	¹ Single infusion
Li <i>et al</i> ^[34]	Prospective?	17 24	IFX	CRP Clinical response Endoscopic healing	Changes in percentages of Foxp3(+) Tregs (mucosal and systemic)
McDermott <i>et al</i> ^[30]	Retrospective	23 (86% infliximab experienced)	ADA	Failure (discontinuation of ADA) Colectomy (follow-up 22 mo)	¹ Short-term failure (increased risk for colectomy)
Olsen <i>et al</i> ^[24]	Retrospective	59	IFX	UCDAI	Mucosal TNF-α mRNA expression
Oussalah <i>et al</i> ^[14]	Retrospective	191	IFX (≥1 infusion)	Primary non-response Colectomy Infliximab optimization Hospitalization (median 18 mo)	¹ Indication for acute severe colitis Hb ≤ 9.4 g/dL Non-response
Park <i>et al</i> ^[15]	Retrospective	89	IFX	Clinical response Clinical remission Colectomy	¹ Mayo score ≥ 11 CMV infection (within prior 3 mo)
Reinisch <i>et al</i> ^[17]	Prospective (UL-TRA 1)	390 (anti-TNF naïve)	ADA	Clinical remission at week 8	¹ Mayo score ≥ 10 CRP = 10 mg/L
Rismo <i>et al</i> ^[25]	Prospective	74	IFX	UCDAI	Mucosal gene expression signature (Th1 and Th17 related cytokines)

Rostholder <i>et al</i> ^[38]	Retrospective observational	56	IFX	Clinical remission	Escalation of infliximab therapy
Sandborn <i>et al</i> ^[16]	Prospective (ACT1&2)	630	IFX	Colectomy (54 wk)	¹ Concomitant steroids CRP ≥ 2 mg/dL Disease duration < 3 yr Mayo ≥ 10 Trough levels
Seow <i>et al</i> ^[40]	Cohort	115	IFX	Clinical remission Endoscopic improvement Colectomy	
Steenholdt <i>et al</i> ^[41]	Retrospective	106 (CD/UC)	IFX	Loss of response	¹ Trough levels Anti-infliximab antibodies
Taxonera <i>et al</i> ^[29]	Retrospective	30 (IFX experienced)	ADA	Clinical response at week12 Colectomy (follow-up 48 wk)	Short-term response at week-12 (Associated with less withdrawal and colectomy rates)
Toedter <i>et al</i> ^[33]	Prospective (ACT-1)	48	IFX	Clinical response	Mucosal gene expression signature

¹Italics correspond to prognostic factors for adverse outcome. IFX: Infliximab; ADA: Adalimumab; UCDAI: Ulcerative colitis disease activity index; HACA: Human anti-chimeric antibodies; CRP: C-reactive protein.

to ADA in the short-term. It should be noted, however, that these parameters did not strongly affect the result and their consideration as predictive factors must be cautious.

In all, the majority of studies appear to support the notion that severe UC demonstrates a less favorable response to treatment with anti-TNF monoclonal antibodies. From the pure clinical standpoint, the best candidate for anti-TNF administration may be an outpatient with moderate to severe UC but not severe disease requiring hospitalization, as defined by the criteria of Truelove and Witts.

In addition to disease severity, other clinical parameters may also affect the response to anti-TNF in UC. Ferrante *et al*^[18] studied a cohort of 100 UC patients who were treated with IFX. More than half had extensive disease, were on immunosuppressants and received a single infusion as opposed to the standard induction scheme. Early clinical response was accomplished in 65% of patients. Younger age was associated with a higher percentage of early clinical response (responders: median age 35.7 years *vs* non-responders: 41.6, $P = 0.049$). Different results were obtained by Jakobovits *et al*^[19] who reviewed the records of 30 patients with refractory UC who had received a single IFX infusion over the period 2000-2006. Half of the patients underwent colectomy over a median follow-up period of 140 d. In this cohort, younger age at diagnosis correlated with increased risk of surgery (colectomy: mean age 27.5 years *vs* non-colectomy 38.7 years, $P = 0.016$). In contrast, the indication before starting IFX was not relevant to colectomy rates. The number of patients in this study was too small for definitive conclusions to be drawn. In the analysis of the ACT trials duration of colitis ≤ 3 years strongly increased the risk for colectomy (hazard ratio = 0.36, $P < 0.001$, respectively)^[16]. Finally, disease extent may also affect response to treatment. Gonzalez-Lama *et al*^[20] studied 47 UC patients who were treated with IFX and were followed for a mean duration of 8 mo. Pre-treatment predictive factors were sought: extent of the disease was the only factor that was related to higher response rates

to IFX ($P = 0.02$). Extensive colitis appeared to respond less favorably in the short term in the aforementioned study of ADA as well^[17].

Laboratory indicators: Among the various laboratory biomarkers of inflammation, C-reactive protein (CRP) has been the most extensively applied to clinical practice. The association between CRP and inflammatory activity in UC has not been equally strong as it is for Crohn's disease. Nevertheless, its relevance increases when cases of severe UC are studied. As these are the patients that usually require administration of anti-TNF agents, the predictive value of CRP for treatment efficacy/failure may be increased in this population. Ferrante *et al*^[21] reported on a cohort of 121 UC outpatients treated with IFX and followed for a median of 33 mo. Eighty-one patients (67%) exhibited short-term response and 21 (17%) underwent colectomy. A value of pre-treatment CRP ≥ 5 mg/L was an independent predictor for colectomy (HR = 14.5, $P = 0.006$). Similar results were presented in a study of 134 Korean patients with UC who had received at least one infusion of IFX^[22]. At week 8, 87% and 45% achieved response and remission, respectively. A pre-treatment CRP ≥ 3 mg/dL was predictive of clinical remission at week 8 (OR = 4.77, $P = 0.01$). The association between elevated CRP and less favorable response to anti-TNF was also confirmed in the analysis of the ACT trials^[16]. A baseline CRP ≥ 2 mg/L was significantly associated with increased colectomy risk (HR = 1.73, $P = 0.04$). Of note, several studies found an association between elevated CRP and colectomy^[21]. Therefore, increased CRP may represent a strong marker of inflammation that requires potent treatment and will respond optimally to anti-TNF. Alternatively, CRP may be an indicator of refractory disease.

In the previous Korean study, high pre-treatment hemoglobin was also a predictor of good response to IFX^[22]. Baseline haemoglobin of ≥ 11.5 g/dL was associated with higher probability for remission at week 8 (OR = 4.47, $P = 0.008$). This is in accordance with the study by Oussalah^[14] who reported that pre-treatment hemo-

globin ≤ 9.4 g/dL predicted primary non-response to IFX (OR = 4.35). This occurred in 22% of 191 treated patients who were included in the study. According to Truelove criteria low hemoglobin is an indicator of severe disease, which increases the risk of non-response to IFX. High pre-treatment hemoglobin may reflect the presence of milder disease that responds better to anti-TNF treatment.

Serum albumin concentration may also have prognostic value. A study by Fasanmade *et al*^[23] focused on the association between serum IFX and albumin concentration. Data from 728 patients who participated in two clinical trials were analyzed. A value of serum albumin that was outside the normal range was directly related to trough IFX levels and clinical response. Patients with low serum albumin had reduced IFX concentration and worse clinical outcomes. This correlation may reflect a common clearance pathway for albumin and anti-TNF antibodies that belong to the IgG class of immunoglobulins. In all, measurement of albumin before commencement of treatment may serve as a predictive marker of the drug's pharmacokinetics.

Immunological and genetic markers: In recent years significant advances have taken place in our understanding of the immunopathogenesis of UC. In addition, genome wide association studies have discovered polymorphisms which confer susceptibility to or protect from developing UC. These studies led to the identification of several immunological markers which may serve as indicators of disease activity and severity. The possibility that such markers may also serve as predictors of response to treatment, in particular to therapy with anti-TNF monoclonal antibodies, has been increasingly explored.

One of the classical immunological markers that are associated with UC is the presence of perinuclear antineutrophil cytoplasmic antibodies (p-ANCA). In two recent studies absence of this marker was strongly associated with better response to IFX. In a retrospective study of 90 patients who were evaluated up to week 14 on scheduled IFX infusions, negativity for p-ANCA (along with disease severity and IL23R genotype) was predictive of IFX efficacy^[13]. Similar results were obtained in the study by Ferrante *et al*^[18]. The authors followed 100 UC patients treated with IFX (84 patients received a single infusion). ANCA seronegativity served as predictor of good response. Notably, a serological phenotype of ANCA+/ASCA- status was particularly correlated with lower rates of response ($P = 0.049$).

During acute flares of UC an abundance of inflammatory mediators are upregulated at the intestinal mucosa and can be detected at both the mRNA and the protein level, whereas, anti-inflammatory treatment is paralleled by a decrease or even disappearance of these markers. Therefore, such markers may hold predictive value for the response to anti-TNF treatment. A first obvious target has been TNF itself. Olsen *et al*^[24] looked for predictive factors of response to induction treatment (weeks 0, 2,

6) with IFX in a cohort of 59 patients with moderate to severe disease. The outcome was assessed based on UC disease activity index (UCDAI). Among various parameters elevated pre-treatment mucosal TNF- α expression was the only independent predictive factor of clinical and endoscopic remission ($P = 0.01$ and $P = 0.003$, OR = 2.5 and 4.8, respectively).

UC-related intestinal inflammation has been characterized by upregulation of several components of the major adaptive immunity pathways (Th1, Th2, Th17). A recent study looked at the expression of the pivotal Th1 (IFN- γ) and Th17 (IL-17) cytokines before and after treatment with IFX^[25]. Mucosal cytokine profile was determined by PCR and confirmed by immunohistochemistry in biopsies of 74 UC patients. Efficacy was evaluated after 3 infusions and was based on UCDAI. High pre-treatment mucosal expression of IL-17 and IFN- γ significantly correlated with remission after induction therapy (OR = 5.4, $P = 0.013$ and OR = 5.5, $P = 0.011$, respectively).

In a much broader approach, Arijis *et al*^[26] performed a gene-array study in mRNA from colonic mucosal biopsies obtained from UC patients who received induction therapy with IFX. Analysis of the arrays revealed genes that were differentially expressed among responders and non-responders. Genes that showed a highly differential expression were osteoprotegerin, stanniocalcin-1, prostaglandin-endoperoxide synthase 2, interleukin 13 receptor alpha-2 and interleukin 11. The sensitivity and specificity in predicting response to IFX based on this gene profiling was 95% and 85%, respectively.

The effect of genetic polymorphisms to response to treatment remains unknown. In the aforementioned study by Jurgens the effect of UC-associated, IL-23R variants on the efficacy of IFX was reported^[13]. In this study of 90 patients, homozygosity for the IBD-risk-increasing IL23R variants was associated with higher probability to respond to IFX than homozygosity for IBD-risk-decreasing IL23R variants (74.1% *vs* 34.6%; $P = 0.001$).

Treatment-related factors: Several studies have shown that the pharmacological history plays an important role in the response to anti-TNF treatment. In the study by Ferrante *et al*^[21], 121 UC patients received IFX and were followed-up for a median of 33 mo. Colectomy was performed in 21 patients (21%). Previous *in* treatment with steroids and/or cyclosporine significantly increased the risk for colectomy (HR = 2.4, $P = 0.033$). A similar association was seen in the study by Oussalah *et al*^[14]. Previous use of cyclosporine was a positive predictive factor for colectomy (hazard ratio = 2.53). Finally, in the analysis of the colectomy rates in the context of the ACT-trials patients who were on steroids when IFX was started had an increased risk for surgery (HR = 1.84, $P = 0.01$)^[16]. However, caution is required for the interpretation of these associations, which should take into consideration the severity of the disease. Indeed, in all of these studies

more severe disease was associated to adverse outcomes and less favorable response to anti-TNF. Therefore, the use of *iv* steroids and/or cyclosporine may simply reflect severe disease.

The association between exposure to immunosuppressants and efficacy of anti-TNF therapy merits special attention. Converging lines of evidence indicate that immunosuppressant-naïve patients respond better to anti-TNF. The efficacy of IFX was evaluated in a cohort of 126 steroid-dependent patients^[27]. Approximately half of the patients achieved steroid-free remission, whereas mucosal healing at 12 mo was accomplished in one third. Thiopurine-naïve status was positively associated to steroid-free remission as well as mucosal healing at 12 mo (HR = 2.8 and OR = 3.6, respectively). In the aforementioned Korean study^[22] immunomodulator-naïve status was an independent predictors for early clinical remission (OR = 4.89, $P = 0.01$). This consistent finding is in agreement with the growing evidence regarding earlier introduction of biologics in patients with moderate disease, as patients who never received thiopurines may have suffered a shorter disease course.

Finally, for patients who receive ADA as a second anti-TNF monoclonal antibody, the treatment efficacy is affected by the response to prior treatment with IFX. This was shown in a recent retrospective study that evaluated the clinical response and colectomy rate in a cohort of 48 UC patients treated with ADA^[28]. The majority (81.3%) was previously exposed to IFX. Early response to ADA at week 12 was significantly more frequent in patients who achieved remission on prior treatment with IFX ($P = 0.01$).

Prognostic factors during anti-TNF treatment

Several recent studies have provided evidence to support the notion that patients with early response to anti-TNF (*i.e.*, within 3 mo) are the ones who will also benefit in the long-term. Early response was defined by a variety of clinical and biological markers in these publications.

Clinical parameters: A Spanish study evaluated the efficacy of ADA in 48 UC patients who were followed-up to week 54^[28]. In this cohort the only predictive factor for colectomy was the absence of early clinical response, which was determined by partial Mayo score at week 12 (colectomy: 14.7% *vs* no colectomy: 42.9%, $P = 0.035$).

These results were replicated in a cohort of 121 UC outpatients^[21]. Eighty-one patients initially responded to IFX with 2/3 maintaining clinical response throughout follow-up. Twenty-one patients ended up with colectomy after a median follow-up of 33 mo. No predictors for durable response were identified. Colectomy on the other hand strongly correlated with early non-response to IFX (HR = 10.8, $P < 0.001$).

In the study by Lee *et al*^[22], 45% of 134 patients with UC who received at least a single IFX infusion, achieved remission at week 8. Short-term remission rates were higher in patients who responded very early, at week 2

(OR = 20.54, $P = 0.006$).

The value of ADA in 30 UC patients who had failed IFX was studied retrospectively^[29]. Response and remission rates were assessed at weeks 4 and 12 and colectomy rates over a mean follow-up of 48 mo. In the long-term 50% were still on ADA and 20% underwent colectomy. The risk of surgery was higher for patients who did not achieve response at week 12 ($P = 0.001$).

Similarly, Mc Dermott *et al*^[30] studied 23 patients who received ADA induction and maintenance treatment. Of note, 86% had previously failed IFX. Discontinuation of ADA over a follow-up period of 22 mo was the primary endpoint and occurred in 70% of patients. Colectomy-free survival at 24 mo was 59%. The only factor associated with increased risk for surgery was the absence of early response to ADA. Among patients who underwent colectomy, 55% had failed ADA at week 12.

Armuzzi *et al*^[31] evaluated the short- and long-term effects of ADA in 88 UC patients out of whom 78% had previously received IFX. The rates of clinical remission increased from 17% to 43% at weeks 4 and 54, respectively. Interestingly, achievement of early remission as well as low CRP at week 12 predicted remission at week 54 (OR = 4.17 and 2.63, respectively).

Laboratory indicators: The same conclusion regarding the predictive value of early response was obtained when laboratory markers of inflammation were studied. We already mentioned the predictive value of low CRP at week 12 in the study by Armuzzi^[31]. In another publication from the same group regarding 126 steroid-dependent patients who received IFX^[27] drop of serum CRP value to normal after the induction-regimen predicted steroid-free remission and mucosal healing at 12 mo (HR = 4.6, OR = 6.0, respectively). Similar results were reported in a study that used fecal calprotectin as an inflammatory marker. Serial weekly measurements of fecal calprotectin were performed in a cohort of 53 patients who received IFX^[32]. Two thirds of patients achieved endoscopic remission at week 10, whereas the median calprotectin level significantly drop from baseline ($P < 0.001$). Early reduction of calprotectin at week 2 predicted endoscopic remission. At week 10, clinical and endoscopic remission strongly correlated to fecal calprotectin concentration.

Immunological markers: Early post-IFX changes of the mucosal and peripheral immunophenotype of UC patients showed strong correlation with clinical response to the drug. Toedter *et al*^[33] studied 113 colonic biopsies from 48 patients who participated in the ACT-1 trial. Biopsies were taken before and after treatment with IFX up to week 30. Gene expression profiling was performed. The investigators were able to identify certain genes that demonstrated significant alterations in patients that responded to treatment with IFX but not in non-responders.

In a study that included both Crohn's and UC, the

effect of IFX on the percentages of regulatory T cells (Treg) was investigated^[34]. Flow cytometry, PCR and immunohistochemistry were applied to quantify the expression of Forkhead box protein3 (Foxp3)-positive T cells in both peripheral blood samples and mucosal biopsies before and after IFX treatment. Responders to IFX were characterized by significantly increased numbers of CD4(+) CD25(+) Foxp3(+) Treg and CD4(+) CD25(-) Foxp3(+) Tregs in blood ($P < 0.05$) and a significant down-regulation in the tissue ($P < 0.001$). The duration of clinical response to IFX correlated to a sustainable peripheral increase of Foxp3 (+) Treg cells.

Although such individual molecular characterization is far from being clinically applicable, it shows that personalized therapy which will be based on the particular immunophenotype may guide the therapeutic approach in the future.

Endoscopic findings: In recent years, mucosal healing (*i.e.*, the disappearance of visible active inflammatory lesions in endoscopy) has emerged as a definitive endpoint in the natural history of UC and an indispensable therapeutic target both in clinical trials and “real-life” practice. This is because mucosal healing has been shown to be associated with sustained long-term remission in patients with UC^[3].

In the pivotal ACT trials endoscopic evaluations were performed at various time points and mucosal healing was defined as Mayo subscore of 0 (normal) or 1 (mild). Early endoscopic improvement at week 8 was associated to improved clinical outcomes^[3]. Accordingly, low endoscopy subscores at week 8 predicted reduced risk of colectomy through week 54 ($P = 0.0004$) as well as higher remission and steroid-free remission rates ($P < 0.0001$).

A single IFX infusion or placebo was administered to 45 patients with acute, steroid-refractory UC^[35]. Three years later the beneficial effect of the drug persisted as less patients in the IFX group underwent operation (50% *vs* 76%, $P = 0.012$). Endoscopic remission at month 3 strongly predicted a reduced long-term risk for colectomy ($P = 0.02$).

Mucosal healing was also a positive predictive factor for long-term remission in the study by Lee *et al*^[22]. A variety of predictors for short-term outcome were identified whereas the only parameter associated with sustained long-term benefit was endoscopic remission (OR = 4.66, $P = 0.04$).

Treatment-related factors: The number of IFX infusions was associated with improved sustained response to anti-TNF treatment. Kohn *et al*^[36] studied the effect of IFX treatment in 83 patients with severe steroid-refractory UC. Patients received ≥ 1 infusions and were followed for a median of 23 mo. Twelve out of 83 patients (15%) had a colectomy within 2 mo. The risk for a prime adverse event was significantly higher among patients who received a single IFX infusion as opposed

to those who were given two or more doses (OR = 9.53, $P = 0.001$).

The combined administration with immunosuppressants appears to have an advantage in comparison to single IFX therapy. This was shown in the study by Armuzzi *et al*^[27]. In this cohort of 126 steroid-dependent UC patients combination treatment with IFX and thiopurines was a predictor of steroid-free remission (HR = 2.2). In another prospective trial Panaccione studied 231 patients with moderate disease who were biologics-naïve and had not received azathioprine over the 3 mo before enrollment. Patients were offered IFX monotherapy, azathioprine monotherapy or combination treatment. Steroid-free remission at week 16 was significantly more common in the combination arm of the study ($P < 0.05$ compared to both monotherapies)^[37].

The need for escalation of anti-TNF therapy is also a poor prognostic factor for long-term outcome. In a cohort of 56 patients with moderate colitis who were treated with IFX, 89% proceeded to maintenance treatment^[38]. During a mean follow-up of 38 mo, clinical remission was achieved in 36% of patients at 12 mo, whereas 54% required escalation of treatment. Intensification of IFX treatment was a negative predictive factor of remission at 12 mo ($P = 0.01$). In accordance, colectomy was performed more often in the “escalation” group (33% *vs* 21%).

In a related study, Cesarini *et al*^[39] showed that rapid response to escalation treatment has a favorable effect on long-term outcome. They studied the records of 41 UC patients with loss of response to IFX who were treated with either dose doubling or interval shortening. The primary outcome was rapid response which was evaluated at the follow-up visit after treatment escalation. Remission and colectomy were evaluated by week 52. The majority (90%) responded rapidly and 46% achieved rapid remission. Only 4 patients (9.8%) underwent colectomy by week 52. The main predictor for avoidance of colectomy was initial response to intensification treatment ($P = 0.002$).

Recent developments emphasize the importance of serum trough levels of IFX and ADA and the formation of antibodies against anti-TNF monoclonal antibodies for the pharmacokinetics as well as the therapeutic efficacy of these drugs. In a study of 115 UC patients on maintenance treatment, clinical outcomes were associated to IFX trough levels^[40]. Detectable drug in serum predicted clinical remission and endoscopic improvement at week 54 ($P < 0.001$ for both parameters). Reduced trough levels correlated with increased risk of colectomy in this cohort ($P < 0.001$). Interestingly, antibody-status was not predictive of response to IFX treatment.

Steenholdt *et al*^[41] retrospectively studied 106 IBD patients on IFX, who either maintained or lost their response. Significantly higher IFX levels and lower antibodies titer were measured in patients with sustained response to IFX ($P < 0.0001$). Moreover, the authors suggested threshold values for the two parameters to ac-

curately predict and/or explain loss of response to IFX.

Similarly, Ben-Horin *et al*^[10] tested the samples of 62 mixed IBD patients for anti-IFX antibodies and serum trough levels. Low trough levels and high antibodies titer were found in 83% of patients with loss of response and in 8% of patients who maintained remission ($P < 0.001$).

Critique of available markers

As the number of UC patients who have been exposed to anti-TNF monoclonal antibodies steadily increases, more factors will be reported that may be associated with better or worse response to these medications. Before, however, their use is recommended for the selection of patients in clinical practice, careful analysis of the specifics of each marker should be performed and inherent problems with the interpretation of the results from clinical trials should be kept in mind.

Clinical markers have the advantage to be readily available and identifiable in a straightforward fashion. They are easy to use, replicable, non-invasive and, overall, convenient for use in clinical practice. Caution, however, is needed when data from clinical trials are analyzed as the definition of a certain parameter may vary between different studies. In particular, clinical response and remission may be related to a variety of activity scoring systems or arbitrarily defined clinical criteria. In addition, the time point in which a certain clinical marker is reported is of pivotal significance. This is so because UC is a lifelong condition and, therefore, only time points with significant length are relevant to a true remission. Criticism also occurs regarding RCTs in the means that they may not always include patients that reflect 'real-life' IBD populations^[42].

Endoscopic markers such as mucosal healing are of significance as recent studies have shown that they are indeed associated with better disease outcomes. It should be noted, however, that the major clinical trials have defined mucosal healing as Endoscopy Mayo score of 0 or 1. Whether the latter score truly represents absolute and complete elimination of inflammation is questionable. In addition, such markers require the performance of an invasive procedure (colonoscopy) soon after the commencement of treatment (≤ 3 mo), which may not be easily acceptable from a patient, in particular when clinical remission has taken place.

Serological markers such as CRP are also easy to obtain. Nevertheless, there has not been good correlation between CRP and clinical activity of UC with the exception of severe cases. In addition, its prognostic value has only been reported in a minority of trials, given the fact that CRP is usually determined in every case of UC. Fecal calprotectin is a good indicator of ongoing acute (neutrophilic) inflammation in the colon. However, no studies have indicated that the magnitude of pre-treatment fecal calprotectin predicts the response to anti-TNF. In addition, the measurement of fecal calprotectin is not widely applied in practice and technical issues exist

regarding the standardization of methodology. It should be noted, however, that both serum CRP and fecal calprotectin may be more useful when their short-term change in response to anti-TNF is considered rather than their absolute pre-treatment values.

Immunological and genetic markers are important as they hold promise for individualized therapy based on the specific characteristics of each individual patient. The major drawbacks for the application of such markers are technical challenges and lack of replication for most results. An additional problem is the redundancy of the immunological pathways that underlie inflammation in UC. Therefore, a single marker may not be sufficient enough to cover the whole mechanism of injury. Similarly, UC is a polygenetic trait and single gene polymorphisms do not usually lead to the manifestation of the disease phenotype. Nonetheless, as additional biological drugs will become available for the treatment of UC, selection of patients according to the predominant immunogenetic pathway may become the most cost-effective approach.

CONCLUSION

Currently, no single marker fulfils all criteria for being an appropriate prognostic indicator for response to anti-TNF treatment in UC. The ideal predictor should be clearly defined, simple and easy to obtain, as well as of repetitive association between different trials. Alternatively, a predictive model which includes clinical, laboratory and even genetic and/or immunological parameters may be more difficult to develop but more accurate in its predictive value. In that context, and whilst our experience with anti-TNF therapy in UC expands, it is important to continue the search for optimal predictive factors of response or failure. Each of the proposed prognostic parameters should be validated in large populations of patients and across clinical trials of different ethnicities. Eventually, personalized treatment may be the best, safest and most cost-effective strategy in diseases with such a complex pathogenetic background.

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Genetic update on inflammatory factors in ulcerative colitis: Review of the current literature

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summarizes the current literature on inflammation-related genetic polymorphisms which are associated with UC. We performed an electronic search of Pubmed Database among publications of the last 10 years, using the following medical subject heading terms: UC, ulcerative colitis, inflammation, genes, polymorphisms, and susceptibility.

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Key words: Ulcerative colitis; Inflammatory factors; Genes; Polymorphisms; Susceptibility

Core tip: Ulcerative colitis (UC) is a disorder of the idiopathic and chronic inflammation of the colonic mucosa. Several genetics factors influence the development of the disease, especially interleukin and interleukin receptor gene polymorphisms and other inflammation-related genes. In this review we collected the current literature on PubMed Database about those genetic markers that are associated with UC, we focused on the following terminology: UC, inflammation, genes, polymorphisms, susceptibility.

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Abstract

Ulcerative colitis (UC) is one of the main types of inflammatory bowel disease, which is caused by dysregulated immune responses in genetically predisposed individuals. Several genetic factors, including interleukin and interleukin receptor gene polymorphisms and other inflammation-related genes play central role in mediating and modulating the inflammation in the human body, thereby these can be the main cause of development of the disease. It is clear these data are very important for understanding the base of the disease, especially in terms of clinical utility and validity, but summarized literature is exiguous for challenge health specialist that can used in the clinical practice nowadays. This review

INTRODUCTION

Ulcerative colitis (UC; MIM 191390) and Crohn's disease (CD; MIM 26600) are the two main, related forms of inflammatory bowel disease (IBD) which are chronic, relapsing inflammatory disorders of the gastrointestinal tract^[1]. The highest annual incidence rate of UC was re-

ported in Europe (24.3/100000) and in North America (19.2/100000). However, in Asia and in the Middle East the rate is much lower (6.3/100000) believed to be associated with the different level of industrialization^[2]. UC has a bimodal pattern of incidence, with the mean age diagnosis between ages 15 and 30 years, and a second smaller peak between ages 50 and 70 years^[3]. Clinically, UC is characterized by superficial, continuous mucosal inflammation and ulcers restricted to the colon, whereas CD is a segmental, transmural disorder involving any part of the gastrointestinal tract^[4].

Although the precise etiology of IBD still remains obscure, the accepted hypothesis is that in genetically susceptible individuals the commensal luminal flora trigger an inappropriate, overactive mucosal immune response causing intestinal tissue damage that is further modified by specific environmental factors (*e.g.*, smoking)^[5].

At first, observational family studies and twin studies directed the interest to genetic components in the pathogenesis of IBD^[6,7]. Recently, genome-wide association studies (GWAS) have resulted in the identification of many novel single nucleotide polymorphisms (SNPs) for CD initially and latterly for UC which is thought to be more genetically heterogeneous than CD. To date, the number of known risk loci has expanded to 163, of which 110 confer common susceptibility to IBD, whereas 30 seem to be specific to CD and 23 to UC^[8].

Immunologically, CD is associated with a T helper type 1 (Th1)^[9] and T helper type 17 (Th17)^[10] immune response, thus interferon gamma/interleukin-12 (IFN γ /IL-12) and interleukin-23/interleukin-17 (IL-23/IL-17) cytokines assign the downstream release of complex network of further pro-inflammatory cytokines (*e.g.*, IL-18, IL-2, IL-1, IL-21, IL-22, IL-17A, IL-17F, IL-26). However, UC is thought to be the result of a Th17 (IL-17) and a modified Th2 response (IL-13, IL-5 and IL-9). In addition, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF α) are produced by both T helper type 2 (Th2) cells and Th1 cells.

The IBD-associated loci encode for genes involved in maintenance of epithelial barrier integrity, antigen pattern recognition, autophagy, innate immunological response, coordination of adaptive immune responses and leukocyte recruitment (Figure 1).

Most of the difference at molecular level between UC and CD is found in human leukocyte antigen (*HLA*) Class II genes and in genes related to pattern recognition [*e.g.*, nucleotide-binding oligomerization domains (*NODs*), *toll-like receptors* (*TLRs*)], innate immunity (*e.g.*, IL-23R) or autophagy pathways (*e.g.*, *ATG16L1*, *IRGM*). The *HLA* class II genes *DR2*, *DR9*, and *DRB1*0103* were identified as susceptibility genes for UC, whereas *DR4* was a protective gene^[11,12]. *HLA* haplotype *DRB1*0103* is significantly associated with disease susceptibility, extensive disease, and an increased risk of colectomy^[13]. While several genes involved in bacterial sensing [nucleotide-binding oligomerization domain

2/caspase activation recruitment domain 15 (*NOD2/CARD15*)] and processing mechanisms (autophagy related genes *ATG16L1* and *IRGM*) are defective only in CD, the Th17/IL-23 axis related cytokines [*e.g.*, IL-23R, IL-12B and their downstream components signal transducer and activator of transcription 3 (STAT3), janus kinase 2 (JAK2)] have been associated with both CD and UC.

Dysfunction of the barrier integrity, enhanced permeability is also a main feature in UC. Recently, in a large review epithelial barrier genes were discussed in detail, namely, extracellular matrix protein 1 (*ECM1*), cadherin type 1 (*CDH1*), hepatocyte nuclear factor 4, alpha (*HNF4 α*), and laminin beta 1 (*LAMB1*).

These genes were found not to be associated with CD, implying they may confer susceptibility specifically to UC^[14]. Interestingly, the *CDH1* locus represents the first genetic association also identified in a GWAS for colorectal cancer susceptibility^[15,16].

In our review we focus on inflammation-related genes and polymorphisms including interleukin and interleukin receptor gene polymorphisms which are involved in the pathogenesis of UC.

INFLAMMATION-RELATED GENETIC FACTORS

Cytotoxic T-lymphocyte antigen 4

Cytotoxic T-lymphocyte antigen 4 (CTLA4) is an inhibitory receptor expressed by activated T cells. It is an important downregulator of the T cell activation and might contribute to peripheral tolerance. CTLA4 is a good candidate gene for susceptibility to UC, because it acts as a negative regulator of T cell activation and T/B, T/monocyte-macrophage cognate interaction. The localization of *CTLA4* gene is on chromosome 2q33. Several genetic polymorphisms have been reported in the human *CTLA4* gene^[17,18].

In a Tunisian population study, where A+49G was analyzed comparing the UC patients with the control subjects, the frequencies of the +49A allele and the homozygous +49 A/A genotype were higher in UC patients than in controls, but those differences were not statistically significant^[17].

In a Dutch Caucasian and in a Han Chinese UC cohort studies the C-318T and A+49G polymorphisms of *CTLA4* gene were examined. No significant differences were observed in distribution of allele, genotype and haplotype frequencies between UC and control group^[19].

A Hungarian cohort was examined for the same polymorphisms and no association was found between heterozygous AG genotype, homozygous GG variant, and G allele frequency of the *CTLA4* gene A+49G polymorphisms comparing the UC (IBD) group to the healthy controls. The A+49G does not represent an obligatory susceptibility factor for UC^[20].

The A-1661G and the T-1722C two other SNPs in the non-exonic region were investigated in the Han

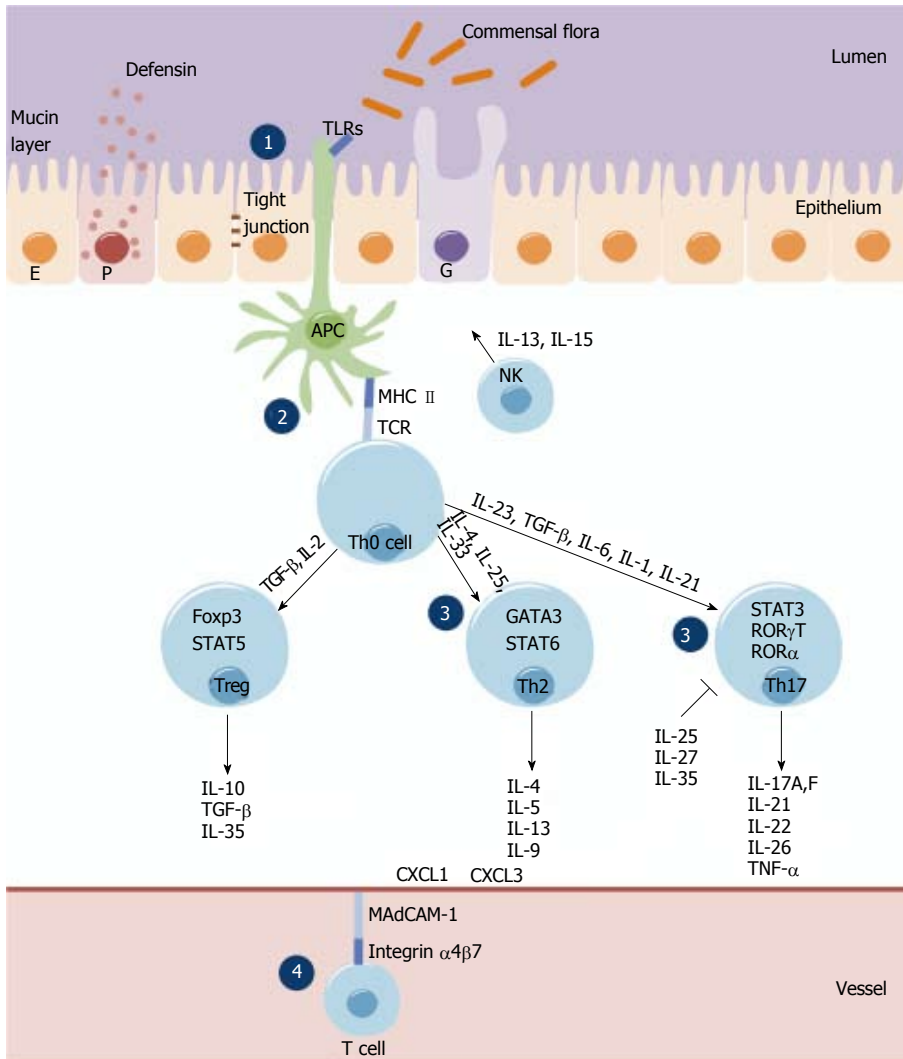


Figure 1 The Ulcerative colitis-associated risk loci. Damage of the epithelial barrier (E), the mucinous biofilm layer secreted from goblet cells (G), the antibacterial peptides (*e.g.*, defensins) produced by Paneth cells (P) and the tight junctions leads to increased permeability in ulcerative colitis (UC). Antigen presenting cells (APCs) (*i.e.*, macrophages and dendritic cells) in the lamina propria are increased in absolute number in UC, they bind microbial products through detection molecules of the innate immune system, including Toll like receptors (TLRs) on the cell surface and on the cytoplasmic NOD-like receptors (NLRs). Stimulation of these receptors induces intracellular signaling cascades, resulting in secretion of large number of cytokines, chemokines, and immunomodulatory factors. The development of the Th2, Th17 and Treg subsets from naïve, Th0 cells during primary immune response is mainly determined by cytokines and chemokines, and is under the control of certain transcription factors: T-bet (T-box expressed in T cells), GATA3 (GATA binding protein), ROR γ t (retinoid-related orphan receptor γ t), ROR α , STATs (signal transducer and activator of transcription) and FoxP3 (forkhead box P3). Leukocyte migration and recruitment from vessels is mediated by selectins, integrins, ICAMs and chemokines (*i.e.*, c-c motif chemokine ligand, CXCL). The UC-associated loci encode genes involved in: (1) maintenance of epithelial barrier integrity (*e.g.*, EBM1, CDH1, HNF4A, LAMB1, PTGER4, SLC22A4/SLC22A5, MYO9B, MDR1); (2) antigen pattern recognition (*e.g.*, NLRs, TLRs); (3) innate and adaptive immunological responses (*e.g.*, IL-23R, IL-12B, TNF α , IL10R, JAK2, STAT3, HLA-region); and (4) leukocyte recruitment (integrin α 4 β 7, ICAM-1, MAdCAM-1, CXCLs, CCRs).

Chinese population. The frequency of A/G + G/G genotype at position -1661 was statistically higher in UC patients than in healthy controls. The G allele frequency was also significantly increased in UC patients than in the controls. The A-1661G polymorphism of the *CTLA4* is a risk factor for UC in Han Chinese of central China. They found no association between T-1722C polymorphism and UC^[18].

Janus kinase 2

Janus kinase 2 (JAK2) is a member of a family of tyrosine kinases involved in a specific subset of cytokine receptor signaling pathways. JAK2 has been found to be

constitutively associated with the prolactin receptor and required for responses to gamma interferon^[21-23].

In GWAS studies several UC loci were identified. The rs10758669 and the rs10974944 SNPs in the *JAK2* locus were found to be strongly associated with UC in the population from the United Kingdom^[14] and Netherlands^[24].

In a Korean population two SNPs (rs10758669 and rs10975003) were investigated. The rs10758669 showed no significant differences in genotype and allele distribution between UC patients and controls, while it was significant on level of genotype and allele frequencies in case of rs10975003. The rs10975003 SNP plays role in

the pathogenesis of UC in Koreans^[25].

Signal transducer and activator of transcription 3

This protein is a member of the signal transducer and activator of transcription (STAT) protein family. It is encoded by the signal transducer and activator of transcription 3 (*STAT3*) gene. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein is activated by phosphorylation in response to various cytokines and growth factors including interferons (IFNs), epidermal growth factor (EGF), interleukin-5 (IL-5), IL-6, hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF) and bone morphogenetic protein 2 (BMP2). STAT3 relays the expression of a variety of genes in response to cell stimuli, and thus plays pivotal role in several cellular processes, such as cell growth and apoptosis^[26-28].

In a large GWAS study the rs12948909 SNPs in the *STAT3* locus was identified and found to be strongly associated with UC in the United Kingdom population^[14].

In the Hungarian population the *STAT3* rs744166 was investigated. *STAT3* rs744166 TT genotype and T allele frequencies were significantly higher in patients with UC than in controls. Logistic regression analysis revealed that the TT genotype confers as an increased risk for the development of UC^[23].

In a North American study the same polymorphisms were tested, but no significant differences were found between the UC group and healthy controls^[29].

Tumor necrosis factor alpha

The pro-inflammatory cytokine, tumor necrosis factor alpha (TNF α) has important pathogenic role both in CD and in UC^[30,31]. Through its ability to cause epithelial barrier disruption in colonic epithelial cells^[32,33] it is responsible for tissue damage. The *TNF α* gene can be found at the inflammatory bowel disease 3 (*IBD3*) locus within the major histocompatibility complex (MHC) region. In several studies it has been found as a susceptibility locus for IBD^[34-36]. Level of TNF α is elevated in serum, stools, and inflamed bowel mucosa of patients with IBD^[37-41].

The polymorphism at position -308 is a point mutation, where the presence of G defines the common variant *TNF1*, and A the less common variant *TNF2*. Susceptibility to UC has been positively^[42] and negatively^[43] associated with carriage of *TNF2* allele. Some studies suggested that this allele might have a small but significant association with greater levels of TNF transcription^[44, 45]. However other authors did not find any influence of *TNF α* bi-allelic polymorphism on UC susceptibility, although they reported a higher frequency of the *TNF2* allele in women with extensive disease compared with those with distal colitis^[46]. In Mexican Mestizo UC patients increased frequency of *TNF2* allele

and *TNF 1/2* genotype was found, suggesting this could be an additional genetic marker for the susceptibility to UC^[47]. Similar findings have been reported in patients with UC with Caucasian origin^[42, 46, 48, 49].

The *TNF α* polymorphisms A-308G and T-857C increase the TNF α production, raising the possibility of correlation with different disease course or response to therapy^[50]. The A-238G and A-308G in *TNF α* promoter region have been found as a susceptibility factor in different autoimmune disorders, including asthma^[51,52], psoriasis^[53] and rheumatoid arthritis^[54]. The polymorphism A-238G was associated with lower production of TNF α in Caucasian UC patients^[46].

In a New Zealand Caucasian UC cohort was found, that carriers of the *TNF α* -308A allele may give higher risk for pancolitis and the necessity for bowel resection^[55]. In Israeli Jewish patients having CD or UC, the allele and carrier frequencies of -857T allele did not differ between IBD patients and controls, suggesting this SNP in Ashkenazi Jewish patients neither determines the susceptibility, nor influences the clinical phenotype of CD or UC^[56].

Different studies supported that *TNF α* -308 in UC may be an ethnic population-specific risk factor. Studies from East Asia suggested strong association of the *TNF α* -308 gene promoter polymorphism for UC in East Asians. Allele frequency of *TNF α* -308A was significantly higher in Han Chinese UC patients than in healthy controls. Haplotype analysis revealed 6 haplotypes including H5 (*TNF* 1031T/863C/857C/380G/308A/238G/) and H3 (*TNF* 1031C/863C/857C/380G/308A/238G/). Haplotype frequency of H5 was significantly higher in UC patients, suggesting that H5 is associated with UC and the *TNF α* gene may be a susceptibility gene to UC^[57]. Interestingly, the meta-analysis did not reveal any association of the *TNF α* -308 gene promoter polymorphism with UC in Europeans^[58]. In a Caucasoid population from the North of Spain the *TNF α* -308 alleles did not influence the appearance of steroid dependency either in UC or in CD^[59]. In Italian pediatric patients the *TNF α* -308 was significantly increased in patients with UC^[60].

In Czech pediatric IBD patients significant differences in *TNF α* -308 A polymorphism were found between UC group and controls, but no differences were noted between this polymorphism and the clinical characteristics of UC^[61].

Significant correlation of the *TNF α* -863A variant was demonstrated with colonic disease and greater height at diagnosis^[62], but in this study they could not find any significant difference for the -857 allele. In patients with UC only a trend toward an increased frequency of steroid resistance was found in carriers of the *TNF α* risk genotype compared to non-carriers^[60].

In the Han Chinese population the *TNF α* C-1031T, A-863C and T-857C allele/carrier frequencies were analyzed between UC patients and healthy controls. They did not find any significant difference of the tested al-

allele/carrier frequencies between UC patients and controls, only the *TNF α* -857T was increased in UC patients but did not reach statistical significance^[57].

Organic cation transporter 1/2

OCTN1 (*SLC22A4*) and OCTN2 (*SLC22A5*) are widely expressed^[63-66], but specifically expressed in principal intestinal cell types affected by CD: epithelial cells, CD68+ macrophages and CD43+ T cells. *SLC22A4* and *SLC22A5* encode the polytopic transmembrane sodium-dependent carnitine and sodium-independent organic cation transporters OCTN1 and OCTN2^[67]. OCTNs have important role in the maintenance of intracellular homeostasis and in the energy production of the cell^[68]. Both OCTNs play important role in the maintenance of gastrointestinal health and in the prevention of gut inflammation^[69, 70].

CD associated variants, the *OCTN1* T1672C and *OCTN2* -207G were as strongly associated with UC in unrelated Caucasian subjects^[71].

Homozygous patients for the *OCTN1* 1672T variant were significantly associated with UC in a study cohort from Italy, suggesting that OCTN1 could have a role in modulating the severity of chronic inflammation in UC^[72].

The mutation that leads to L503F substitution in the OCTN1 protein can alter the transporter's activity^[73, 74]. Only a weak gender-specific effect of L503F was observed at male UC patients in a cohort of familial and sporadic IBD from the central Pennsylvania, United States^[75].

Multidrug-resistant transporter-1

The multidrug-resistant transporter-1 (*MDR1*) gene encodes the transmembrane protein, P-glycoprotein 170 (Pgp)^[76]. This gene is an excellent candidate gene for the pathogenesis of IBD^[77]. Pgp functions as an ATP-dependent efflux transporter pump and is expressed in many normal tissues like in the epithelial surfaces of the intestine, biliary ductules, proximal tubules of kidneys and central nervous system^[78-80].

One of the most significant *MDR1* gene mutations is the C3435T polymorphism. Decreased expression of the *MDR1* gene and lower Pgp activity has been associated with this variant. However, studies showed conflicting results. In a German study the T allele and TT genotype frequencies of C3435T polymorphism were significantly increased in UC patients^[81]. Glas *et al.*^[82] found in a small group of UC patients partial accordance with a trend towards an increased frequency of T allele compared to controls, but a statistical difference was detected only in one of two different control groups. In a meta-analysis significant association of the 3435T allele and the 3435TT genotype has been found with UC^[83]. The triallelic G2677T/A and the C3435T have been shown to correlate with Pgp expression^[84-87]. Significant association of C3435T and G2677T was detected with UC: UC patients had significantly higher frequency of 2677T

allele and of the 3435TT genotype. Haplotype analysis revealed that carriers of 3435T/2677T haplotype have significantly higher risk of having UC^[88]. In a Japanese UC cohort the C3435T was predictive of susceptibility to later onset UC, but not for the early onset of UC^[89].

Large study with German and British UC and CD patients failed to demonstrate association. It was confirmed, that this SNP is associated with UC especially in patients with extensive colitis^[90]. In addition completely negative findings have been reported in large studies from North America^[7], Slovenia^[91] and Italy^[92]. Similarly to these results, UC patients with Caucasian origin from central Poland were found that *MDR1* C3435T polymorphism is not a risk factor for IBD, including both UC and CD^[93].

A study with New Zealand IBD patients supported the role of *MDR1* as a candidate gene for UC. Heterozygous carriers for the variants C1236T, rs2235046 and G2677T/A showed a lower risk of developing UC compared with homozygotes. Subgroup analysis revealed that C1236T and rs3789243 are associated with IBD when stratified for age of onset. The *MDR1* variant rs3789243 was found to be associated with pancolitis in UC patients^[94]. In the genetically heterogeneous North Indian UC cohort was found that this SNP is significantly overrepresented in UC patients.

When German IBD patients were genotyped for the two *MDR1* SNPs in positions 2677G>T/A and 3435C>T it was found that the combined genotypes derived from these positions are possibly associated with young age onset of UC and severe course of disease^[95]. The 2677T allele was significantly increased in British UC cases compared with controls. The TT genotype was significantly associated with severe UC. No significant association was seen with C3435T and UC or any clinical subgroup. A meta-analysis of 9 association studies of C3435T showed a significant association of the 3435T allele with UC, but not with CD. These results indicated that *MDR1* sequence variants are associated with a small increase in the risk of developing UC and may influence disease behavior^[96]. The *MDR1* gene polymorphism G2677T/A showed significant association with CD, and the C3435T with Spanish UC patients^[97]. The *MDR1* 3435 TT genotype and T-allelic frequencies were significantly higher in patients with UC compared with controls. The association was strongest with extensive UC, and this was also confirmed with multivariate analysis. However G2677T was not associated with UC or CD. Two-locus haplotypes showed both positive (3435T/G2677 haplotype) and negative (C3435/2677T haplotype) associations with UC. Homozygotes for the haplotype 3435T/G2677 were significantly increased in UC. Allelic variations of the *MDR1* gene determined the disease extent as well as susceptibility to UC in the Scottish population^[90].

Nucleotide-binding oligomerization domain1/ caspase activation recruitment domain 4

Nucleotide-binding oligomerization domain1/ caspase

activation recruitment domain 4 (*NOD1/CARD4*) is a member of the Nod-like receptor family, which is phylogenetically conserved^[5, 98]. It is constitutively expressed in epithelial cells throughout the gastrointestinal tract^[99]. *NOD1/CARD4* contains leucine-rich repeat (LRR) domain and NOD domains and has only one CARD domain^[100].

Polymorphism in LRR domain of the *NOD1/CARD4* gene showed association with disease severity of UC in North Indian patients. This might be due to disruption of the LRR region critical for NOD1-mediated bacterial sensing. Haplotype-based approach showed that GTTG haplotype carriers were over represented in UC patients which could increase the risk of the disease^[101].

Initially, it was suggested that there is association of the deletion variant of *NOD1/CARD4* +32656 (complex intronic insertion-deletion polymorphism) with susceptibility to IBD using a combination of transmission disequilibrium testing (TDT) and case-control analysis^[102]. However this variant was not associated with a strong effect on susceptibility to IBD in children and adults in a Northern Europe study cohort^[103]. Similar results have been found in the East Anglia IBD cohort, where no association was found between *NOD1* +32656 and IBD and also no heterogeneity between UC and CD^[104].

Toll-like receptors

Essential components of innate immunity are the Toll-like receptors (TLRs). These are transmembrane receptors which recognize the microbial compounds from different bacteria, fungi and viruses^[105-107]. TLRs are expressed by intestinal epithelial cells and immune cells in IBD patients^[108,109]. TLR signaling in the intestinal sites of the colon can inhibit the inflammatory responses and maintains the colonic homeostasis^[110-112]. TLRs can be found on the cell membrane (TLR1, 2, 4, 5 and 9) or on intracellular organelles (TLR3, 7 and 8)^[113]. From the 10 human TLRs we review only three members regarding to their association to UC.

TLR2 is localized on the cell's surface. With its cofactors (TLR1 and TLR6) it binds lipoproteins, which are important surface antigen of the Gram-negative outer membrane^[114]. TLR4 consists of a leucine-rich repeat region (LRR) and an intracellular domain homologous to IL-1 receptor^[115]. It recognizes conserved pathogenic motifs of Gram-negative bacteria, mainly lipopolysaccharides (LPS). Signaling through TLR4 results in the activation of the transcriptional activator, known as nuclear factor κ B (NF- κ B)^[116]. Similarly to TLR2, TLR9 is localized on the cell's surface. It recognizes unmethylated CpG DNA in bacteria and viruses^[117,118].

The allele and carrier frequencies of the Thr399Ile mutation in the *TLR4* gene were significantly associated with UC in a Caucasian population^[119]. Association of *TLR4* Asp299Gly polymorphism with UC was reported first in Caucasian UC patients^[120]. In a study, mentioned before^[119], increased frequency of this polymorphism

was observed, but it did not reach statistical significance. Similarly to Török *et al* study, the Asp299Gly and Thr399Ile mutations in *TLR4* gene were associated with UC in Greek and in North Indian patients^[121,122], but not in Dutch or Italian patients^[123,124]. Interestingly, the *TLR4* Asp299Gly did not show association with UC in different Asian UC populations^[125-128].

The *TLR2* Arg677Trp and Arg753Glu, *TLR4* Asp299Gly and Thr399Ile, and *TLR9* gene C1237T polymorphisms were genotyped in Chinese Han IBD patients; however none of these polymorphisms was associated with IBD. In Caucasians, both TLR4 299Gly and 399Ile conferred as a significant risk factor for developing UC and CD^[127].

Three SNPs of *TLR9* (C-1486T, G1174A, A2848G) were genotyped. These variations were associated with an increased risk of UC in the Japanese population. *TLR9* -1486CC, 1174GG and 2848AA showed increased risk for UC, but *TLR9* -1486TT, 1174AA and 2848GG decreased the risk of UC, although there were no correlations between SNPs and disease phenotype or *TLR9* mRNA expression^[129]. Possible associations between genetic variations in *TLR9* and IBD in the German population were investigated, but no associations were detected between *TLR9* gene variations and UC susceptibility^[130].

Cell adhesion molecules

Cell adhesion molecules (CAM) mediate the extravasation of leukocytes and their accumulation in inflamed intestinal mucosa. This process is controlled by a family of CAM including the intercellular cell adhesion molecule (ICAM-1), the platelet endothelial cell adhesion molecule (PECAM-1), the selectins (E, L, and P selectin) and the integrins^[131,132].

ICAM-1 (CD54) is a cell surface glycoprotein belonging to the immunoglobulin superfamily. It plays key role in transendothelial migration of leukocytes, and lymphocyte activation. The membrane glycoprotein PECAM-1 (CD31) is expressed on vascular endothelial cells, platelets, some lymphocyte subsets, and monocytes^[133-135]. It has important role in transendothelial migration of circulating leukocytes during inflammatory process^[136], apoptosis^[137] and integrin regulation^[138]. The E-selectin (CD62E) is a glycoprotein, which is expressed on endothelial cells in response to pro-inflammatory cytokines (IL-1, TNF). It supports rolling of leukocytes at sites of inflammation and tissue injury. E-selectin expression is upregulated both in CD and in UC patients playing an important role in mediating of the inflammatory process in IBD^[139]. L-selectin (CD62L) is expressed on normal naive T and B cells, leukocytes and on natural killer (NK) cells. It is involved in the adhesion of T cells to endothelial cells, which are regarded as crucial in the selective migration of lymphocytes to inflamed tissue sites during an inflammatory response^[140].

Several mutations in *ICAM1* (G241R and K469E), *PECAM-1* (V125L), *PECAM-1* (G98T and S128R),

E-selectin (L554F) and *L-selectin* (F206L) were analyzed in Tunisian IBD patients and controls. A significant increase in allele frequencies of 206L of *L-selectin* and the associated genotype F/L was observed both in UC and in CD patients. In the subgroup analysis the L206 allele and F/L206 genotype frequencies were significantly increased in UC patients with left-sided type. No significant differences in allele or genotype frequencies were observed for *ICAM-1*, *E-selectin*, and *PECAM-1* polymorphisms between UC patients, CD patients, and controls^[141].

INTERLEUKINS IN UC

Interleukin 1

Interleukin 1 (IL-1) is pro-inflammatory cytokine, which affects cell proliferation, differentiation, and the function of many innate and specific immunocompetent cells, and acts as an endogenous pyrogen. It broadcast many inflammatory diseases by initiating and potentiating immune and inflammatory responses^[142].

IL-1 is composed of two main proteins the IL-1A and the IL-1B^[143]. IL-1B has major role in initiating and amplifying the inflammatory response^[144]. The IL-1 receptor antagonist (IL-1RN) is an anti-inflammatory cytokine, which lacks the IL-1 receptor accessory protein (IL-1RAP) interacting domain^[142].

In Mexican Mestizo UC patients five SNPs were analyzed; the rs419598, the rs315951 and the rs315952 in the *IL-1RN* gene, the rs16955 in the *IL-1B* gene and the 3811058 in the *IL-1F10* gene. Significant increased frequencies of *IL-1RN*6/1TC (rs315952), *IL-1RN*6/2CC (rs315951) and decreased frequency of *IL-1B*-511 TC (rs16944) genotypes were found in UC patients. The patients group showed increased frequencies of *IL-1RN* CTC and TCG haplotypes, whereas TTG and CTG haplotypes frequencies were decreased^[145].

IL-2

IL-2 functions as a T cell growth factor, furthermore it supports the proliferation and differentiation of NK cells to increase their cytolytic functions. This IL plays important role in the development of Th1, Th2, Treg, and Th17 differentiation^[146].

In the *IL-2/IL-21* region several polymorphisms (rs6822844, rs13151961, rs13119723 and rs6840978) were studied. In a Dutch population the minor alleles of the examined SNPs were associated with IBD. The strongest association of these SNPs was found in the UC patients. In an Italian UC cohort the same strong association of the minor alleles was observed with UC. Similarly to this results, in the North American study was demonstrated, that these alleles have the strongest effect among the IBD patients in the UC subgroup^[147].

IL-6

IL-6 is a multifunctional, pleiotropic cytokine that is responsible for regulation of immune responses, acute-

phase responses, hematopoiesis, and inflammation^[148].

An Irish population study the *IL-6* -174 genotype frequency showed significant difference between CD and UC group^[149]. In the Caucasian population the same polymorphism was examined in CD and UC patients and found significant difference UC and CD susceptibility^[150].

IL-8

IL-8 is member of the CXC chemokine family^[151]. Its has two receptors the CXCR1 (IL-8RA) and the CXCR2 (IL-8RB)^[152]. It exerts effect mainly on the chemotaxis and migration of neutrophils, monocytes, lymphocytes, and fibroblasts^[153].

IL-8 T-251A was analyzed in a Polish population. The allele frequency showed significant difference comparing the UC group to the controls^[154], however this association was not observable in a Chinese UC cohort^[155]. Additional polymorphisms were also tested and their effect on the serum level of IL-18. Haplotype frequency of the -353A/-251A/+678T haplotype was considerably higher in UC group compared to controls, suggesting this haplotype is likely to be more common in severe UC patients than in mild to moderate cases^[155].

IL-10

The anti-inflammatory cytokine IL-10 is produced by many cells like monocytes, T cells, B cells, NK cells, macrophages, and dendritic cells (DCs). It prevents the antigen presentation and also the subsequent release of pro-inflammatory cytokines, so it alleviates the activated immune system^[156].

In a GWAS, the polymorphism rs3024505 demonstrated the most meaningful association in the combined verification UC samples, suggesting that defective IL-10 function plays important role in the pathogenesis of the UC^[157]. The same polymorphisms was investigated in Australian population^[158] and Danish cohort^[159] and found that the rs3024505 was associated with the risk of UC.

Three promoter polymorphisms of the *IL-10* gene G-1082A, C-819T, and C-592A were studied in many population but the results are contrary. In an Italian cohort the G-1082A and the C-819T SNPs were investigated. The -1082 genotype frequencies were significantly different between UC patients and controls. The frequency of the -1082A allele was also significantly higher in the UC patients than in controls. Allele and genotype frequencies of T-819C were not significantly associated with the disease. Furthermore, the frequencies of haplotypes -1082A/-819C and -1082A/-819T, which have been described to have a decreased promoter activity, were significantly increased in UC patients than in controls^[160]. In a North-Eastern Mexican population the G-1082A and the C-592A SNPs were examined. The -1082 AA and -592 AA genotypes showed significantly lower frequencies in UC compared to healthy controls, while individuals heterozygous at *IL-10*-1082 have sig-

nificantly increased occurrence of UC^[161]. In a Tunisian group the A-627C and the G-1117A polymorphisms were examined and found that these two variants influencing the UC susceptibility and phenotype^[162]. In the Asian population the association was confirmed between A-1082G polymorphism and UC^[125, 163].

IL-12

IL-12 is an interleukin that is naturally produced by dendritic cells, macrophages and human B-lymphoblastoid cells in response to antigenic stimulation. It participates in the differentiation of naive T cells into Th1 cells and involved in the activities of natural killer cells and T lymphocytes. IL-12 mediates gradation of the cytotoxic activity of NK cells and CD8+ cytotoxic T lymphocytes^[164]. IL-12 consist of two subunit p35 (IL-12A) and p40 (IL-12B), which is shared by IL-12 and IL-23 cytokines. The IL-12 receptor has two subunits: IL-12RB1 and IL-12RB2^[165, 166].

In a German population four SNPs (rs3212227, rs17860508, rs10045431 and rs6887695) of the *IL-12B* were investigated. Two SNPs, the rs10045431 and rs6887695 showed association with increased UC susceptibility^[167]. From these SNPs the rs6887695 was investigated in a Japanese population where significant association was manifested between UC patients and controls^[168].

IL-17

The interleukin 17 (IL-17 or IL-17A) is a pro-inflammatory cytokine secreted by activated T cells its main tasks is inducing and mediating pro-inflammatory responses. It induces the production of many other cytokines, chemokines and prostaglandins from fibroblasts, endothelial cells, epithelial cells, keratinocytes, and macrophages^[169-171].

In a Japanese population the rs2275913 SNP in the *IL-17A* gene and the rs763780 SNP in the *IL-17F* gene were investigated and found significant differences between UC group and healthy controls on level of -197A/A and 7488T/T genotype frequencies^[172].

Even more recently, a GWAS in a very large European UC cohort identified an association between another IL-17 pathway gene (*IL-17REL*) and UC^[173].

IL-18

IL-18 is produced by macrophages and other cells. It functions by binding to the interleukin-18 receptor, and together with IL-12 it induces cell-mediated immunity following infection. IL-18 induces gene expression and synthesis of TNF, IL-1, Fas ligand, and several chemokines^[174].

In the *IL-18* gene several polymorphisms were examined. The G-137C, the C-607A and the G-656T are located in the promoter region, while the A105C, the T113G and the C127T are coding variants. In a Japanese study the G allele at 113 and the T allele at 127 were significantly higher in patients with UC compared to the

control^[175]. In another Japanese study allele and genotype frequency of G-137C were significantly higher in the proctitis-type UC patients than in controls^[176]. The frequency of haplotype 2 (-607A, -137C), which have lower promoter activity and IFN γ -mRNA level was significantly increased in the proctitis-type patients than in the control group^[176]. The C-607A and the G-137C SNPs were associated with the development of UC in Tunisian patients. The -137GG genotype frequency was significantly higher in UC than in controls and statistically significant association was found between -607AA genotype in UC patients and the distal localization of the lesions^[177].

IL-23

IL-23 main functions are very important in innate and adaptive immunity to regulate Th17 function and expansion^[178]. This cytokine induces CD8+ memory T cells to proliferate and produce IL-17. IL-23 binds to its receptor IL-23R, which polymorphisms play the main role in the autoimmune diseases^[179-181] especially in IBD^[182].

Several independent functional SNPs of the *IL-23R* gene and its neighboring region were determined; several were found susceptible to (rs10889677, rs11209032, rs11465804, rs11805303, rs1495965, rs2201841, rs1004819) CD and UC in non-Jewish subjects^[183].

In a Chinese cohort the rs7530511 and rs11805303 SNPs were studied and positive association was found between these variants and UC susceptibility^[184]. In the Jiangsu Han population the rs11805303 was found as a susceptibility factor to UC^[185].

In a Swedish population the rs10889677, the rs11209032, the rs11465804, the rs2201841 and the rs1004819 polymorphisms were investigated, and found that the rs11465804G, rs2201841C and rs1004819T allele frequencies showed significant differences between UC patient group and control. These genetic variants are individual risk factor for developing the disease^[186].

In Hungarian UC patients for the *IL-23R* rs1004819A allele we found significantly higher allele frequency compared to control subjects and the SNP rs2201841 showed significant association with UC risk for homozygotes^[187].

IL-26

Expression of IL-26 seems to be restricted to memory T cells, NK cells, and Th17 cells. Thereby it could have pro-inflammatory effects in IBD^[188].

Only a few markers were investigated, from these the rs2870946-G and the rs1558744-A showed association with UC^[189]. Further meta-analysis study confirmed the association of rs1558744-A with UC^[190].

CONCLUSION

This review shows that substantial progress has been made in the past 10 years in to find inflammatory related genetic factors and cytokines in UC. We reviewed different genes and gene polymorphisms which play role in the inflammatory process of UC. These genes could be

potential targets of novel treatment strategies.

From the reviewed genes contributing to inflammation *TNF α* , *MDR1* and *TLRs* were the most investigated genes. *TNF α* has been found as a susceptibility locus for both UC and CD^[34-36]. The *TNF2* allele and *TNF 1/2* could be good candidate markers for the susceptibility to UC. Based on the different studies with different populations (East Asians, Han Chinese, Spanish Caucasoid, Italian, and Czech), the *TNF α -308* is the most studied SNP and this may be an ethnic population-specific risk factor for UC especially for Asian populations but not for Europeans. It should be noted this polymorphism is also a susceptibility factor in other autoimmune disorders (asthma, psoriasis, and rheumatoid arthritis) too.

The *MDR1* C3435T is one of the most tested SNP in UC, but with conflicting results. Some studies showed significantly increased 3435T allele and 3435TT genotype frequencies of C3435T^[81,83,97], or only a trend towards an increased frequency of T allele^[82], or the T allele was predictive of susceptibility to later onset UC, but not for the early onset of UC^[89]. But some studies failed to demonstrate association with UC^[91-93]. Other SNPs of *MDR1* (C1236T and rs3789243) were associated with IBD when stratified for age of onset. The rs3789243 was found to be significantly overrepresented in genetically heterogeneous North Indian UC patients.

We reviewed 3 members from the 10 human *TLRs* regarding to their association to UC. Allele and carrier frequencies of the *TLR4* Asp299Gly and Thr399Ile were significantly associated with UC in Caucasian^[119,120], Greek and in North Indian patients^[121,122], but not with Dutch, Italian^[123,124] or Asian patients^[125-128]. Interestingly the *TLR9* polymorphisms (C-1486T, G1174A, A2848G) were associated with an increased risk of UC in the Japanese population. *TLR9* -1486CC, 1174GG and 2848AA polymorphisms show increased risk for UC, but *TLR9* -1486TT, 1174AA and 2848GG decrease the risk of UC^[129].

From the reviewed cytokines *IL-10*, *IL-18* and *IL-23* were the most investigated genes. The *IL-10* is a major anti-inflammatory cytokine, which attenuates the activated immune system with inhibiting both the antigen presentation and subsequent release of pro-inflammatory cytokines. *IL-10* is a shared risk gene for CD and UC too. The promoter polymorphisms of this gene (G-1082A, C-819T, and C-592A) which are in tight linkage disequilibrium were extensively studied in many populations but with contradictory results. In the Caucasian population the carriers of G-1082A SNP were more susceptible to UC, whereas in another study carriers were associated with lower UC incidence^[160,161]. In the Asian population the results strengthened the positive relationship between this SNP and UC susceptibility^[125,163]. In a North-Eastern Mexican population the -592AA genotypes showed significantly decreased frequency in UC compared the results to the healthy controls^[161]. In a Tunisian group the A-627C and the G-1117A variants influencing the UC susceptibility and phenotype^[162].

Several other studies handle with these non-coding SNP in CD too and determine susceptibility to the disease or not.

The G-137C, the C-607A and the G-656T promoter SNPs and several others in the coding regions of *IL-18* gene (A105C, the T113G and the C127T) were examined. The Japanese population is the most studies for these SNPs, significant difference was found in the allele frequency of the A105C between CD patients and controls, while this correlation could not be detected in UC patients. The 113G and 127T allele frequencies were significantly increased in patients with UC compared the results to the healthy controls^[175]. In case of promoter polymorphisms, the -137CC genotype frequency was significantly increased in proctitis-type UC patients than in controls, while the other two C-607A and G-137C SNPs were associated with the development of UC in Tunisian patients^[177].

The *IL23R* gene was identified as a CD susceptibility gene in North American non-Jewish subjects. Several independent functional SNPs in the gene and its neighboring region were determined^[183]. After the primary publications, several studies have been published these SNPs in IBD and other autoimmune disease too (ankylosing spondylitis, psoriasis, Sjögren syndrome, systemic lupus erythematosus). From these SNPs (rs10889677, rs11209032, rs11465804, rs11805303, rs1495965, rs2201841, rs100481) several are risk factor to IBD both in European and Asian populations^[185-187].

It can be established that these interleukin gene variants are strongly population dependent but in the given population they can be predictors for CD or UC. Despite the advances in the field of UC/IBD genetics, testing for these genetic variants is currently not recommended for clinical purposes^[191].

Understanding of the detailed pathogenesis of IBD and identifying new disease associated SNPs led to the development of selective inhibitors for ILs, chemokines and their receptors. This strategies can optimize treatment efficacy and lead to personalized medicine based on the patient's genotype.

Biological agents are used in patients with moderate to severe disease activity who have failed conventional therapy with glucocorticoids and thiopurines. Today, the most effective and best studied anti-cytokine agents in IBD are the anti-TNF α antibodies. The mechanism of action of TNF α antagonists is based on the neutralization of both soluble TNF α and membrane TNF α and has a more global effect on inflammation than the blockade of other cytokines. Currently, three TNF α inhibitors are approved by the United States Food and Drug Administration (FDA) for inducing and maintaining clinical remission in UC: the chimeric (25% murine and 75% human sequence) monoclonal full-length IgG1 mAb infliximab^[192], the fully human mAbs adalimumab^[193,194] and golimumab^[195]. The pegylated humanised antibody certolizumab pegol is approved only for CD (beside rheumatoid arthritis, RA and psoriatic arthritis). Etanercept, a

dimeric fusion protein consisting of soluble p75-TNFR2 and the Fc portion of human IgG1, used in rheumatoid arthritis therapy, is not efficient for the treatment of intestinal inflammation^[196].

Despite the expeditious development of newer biological therapies, only few have shown benefit in clinical trials in UC. Targeting of IL-23 or the IL-23 receptor or IL-23 axis is a potential therapeutic approach for autoimmune diseases including psoriasis, IBD, RA and multiple sclerosis^[197]. Recently, testing of anti-IL-12/23 treatment in patients with CD has been performed. In Phase II trial, patients with moderate to severe CD that was resistant to TNF antagonists had an increased rate of response to induction with the fully human mAb ustekinumab directed against the p40, as compared with placebo^[198]. However, due to the common p40 subunit and IL-12RB1 chain, the major drawback of anti-IL-23 treatment can be the simultaneous inhibition of IL-12 and a possible shutdown of the immune system. Nevertheless, it would be much more useful to design drugs that target the IL-23p19 or IL-23RA itself, so inhibiting IL-23 without modifying the effects of IL-12^[197].

Treatment of CD patients with the IL-17 blocker secukinumab (anti-IL-17A) was ineffective and higher rates of adverse events were noted compared with placebo^[199].

One new additional treatment for UC may be tofacitinib, an inhibitor of Janus kinases 1, 2, and 3 with *in vitro* functional specificity for kinases 1 and 3 over kinase 2, which is expected to block signaling involving gamma chain-containing cytokines including ILs-2, 4, 7, 9, 15, and 21. Tofacitinib, was approved for the treatment of RA in the USA, Japan and Russia in April 2013. In a double-blind, placebo-controlled, Phase II trial, patients with moderately to severely active UC treated with tofacitinib were more likely to have clinical response and remission than those receiving placebo^[200].

Targeting leukocyte recruitment and cell adhesion molecules could be also an option for IBD therapy. Natalisumab, a recombinant humanised monoclonal IgG4 antibody, targets both the $\alpha 4 \beta 1$ heterodimer located in the central nervous system and the $\alpha 4 \beta 7$ integrin in the gut. The FDA approved natalizumab for both induction of remission and maintenance of remission for moderate to severe CD, though it has not been approved for this use in the European Union due to concerns over its risk/benefit ratio (risk of progressive multifocal leukoencephalopathy)^[201]. Vedolizumab is a humanized mAb that specifically recognizes the $\alpha 4 \beta 7$ heterodimer, selectively blocks gut lymphocyte trafficking without interfering with trafficking to the central nervous system. In the Phase III study, vedolizumab was more effective than placebo as induction and maintenance therapy for UC suggesting that blockade of T cell homing in the gut may favor mucosal healing in UC^[202, 203].

The exact positioning of these promising new therapies in the management of UC remains uncertain currently. Additional long-term safety data and clinical

experience will be needed to determine an overall benefit/harm ratio of newly developed biological agents.

The identified separate loci in IBD research individually have only modest effects on IBD susceptibility. They account together for only 20%-25% of the heritability, suggesting that gene-gene interactions as well as gene-environmental interactions could play a key role in IBD pathogenesis and fill the so called “genetic vacuum” of polygenic diseases^[204]. More complete understanding of the immunopathogenic role of the various genes and ILs in intestinal inflammation will help in the development of more effective novel therapeutic strategies in UC. Next generation techniques in combination with the data analysis by systems-biology approach hopefully will contribute to the personalized therapy of the patients in the near future.

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Risk of cardiovascular disease in inflammatory bowel disease

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Abstract

Abundant scientific evidence supporting an association between inflammatory bowel disease (IBD) and venous thromboembolic events, caused by an IBD related hypercoagulability, is acknowledged and thromboprophylactic treatment strategies are now implemented in the management of IBD patients. In contrary, the risk of arterial thromboembolic disease, as ischemic heart disease, cerebrovascular events, and mesenteric ischemia in patients with IBD remains uncertain and the magnitude of a potentially increased risk is continuously debated, with ambiguous risk estimates among studies. The evident role of inflammation in the pathogenesis of atherosclerosis forms the basis of a biological plausible link; the chronic systemic inflammation in IBD patients increases the risk of atherosclerosis and thereby the risk of thrombotic events. Further, studies have shown that the burden of traditional risk factors for atherosclerosis, such as obesity, diabetes mellitus, and dyslipidemia is lower in IBD populations, thus further strengthen the role of non-traditional risk factors, as chronic inflammation in the linking of the two disease entities. Likewise, mortality from cardiovascular disease in IBD remains questioned. The aim of the current review is to give an up-date on the existing evidence of the possible

association between IBD and cardiovascular disease and to discuss traditional and non-traditional risk factors.

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Key words: Inflammatory bowel disease; cardiovascular disease; Risk; Ulcerative colitis; Crohn's disease

Core tip: The increased risk of venous thromboembolic events in inflammatory bowel disease (IBD) patients is well-established and prophylactic strategies are implemented in current guidelines. The risk of arterial thromboembolic complications in IBD remains uncertain. Together, the systemic inflammation in patients with IBD and the inflammation-driven development of atherosclerosis form the basis of a potential association between the two disease entities. The present review will provide a summary of the existing literature on the association between IBD and thromboembolic diseases and discuss potential risk and preventive factors.

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INTRODUCTION

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD) are systemic, chronic inflammatory conditions that predominately affect the gastrointestinal tract but are also characterized by numerous extraintestinal manifestations, assumedly caused by concomitant systemic inflammation. It is well-established that the risk of venous thromboembolic event is increased in IBD patients^[1], primarily during flares^[2],

potentially due to an inflammation induced state of hypercoagulability. However, the true magnitude of this risk and the associated mortality rate remains debated.

In the last decade, it has become increasingly evident that chronic systemic inflammation plays a pivotal role in the pathogenesis of atherosclerosis^[3]. Further, the observation of increased thickness of the carotid intimal-media (a measure of atherosclerotic burden), endothelial dysfunction, and atherogenic alterations in the lipid profile of patients with IBD has further fuelled the hypothesis of a potential increased risk of atherosclerosis-driven vascular diseases in IBD^[4-6]. Likewise, an increased risk of cardiovascular diseases (CVD) in other inflammatory conditions as rheumatoid arthritis^[7], psoriasis^[8] and systemic lupus erythematosus^[9] is now established, independent of traditional cardiovascular risk factors. Currently, reported results on risk of CVD in IBD have been ambiguous with studies revealing an increased risk of both ischemic heart disease (IHD) and cerebrovascular accidents (CVA) while others have shown no association^[10-13]. Additionally, a few studies have suggested that IBD patients have a lower burden of some of the traditional risk factors for CVD, such as hypertension, diabetes mellitus, dyslipidemia, and obesity, and that non-traditional risk factors could play an important role for IBD patients^[13,14]. Overall, this has led to an ongoing debate of whether the risk of arterial thrombotic disease is increased in IBD patients, what the underlying mechanisms are, and whether a strategy for disease specific risk assessment should be implemented in the management of IBD patients.

The aim of the current review is to give an update on the existing evidence on risk of atherosclerosis-related vascular disease, including ischemic heart disease, cerebrovascular accidents, mesenteric thrombosis, and venous thromboembolic events and associated risk factors and mortality rates in patients with IBD and further to evaluate on future prospects and preventive factors.

VENOUS THROMBOEMBOLIC EVENTS

The association between venous thromboembolic events (VTEs), comprising deep venous thrombosis (DVT) and pulmonary embolism (PE), and IBD was indicated as early as in 1936 by Bargen *et al*^[15]. In 1986, fifty years after the suggested association, Talbot *et al*^[16] was the first to report valid results on the incidence of VTEs in 7199 IBD patients from the Mayo Clinic, US and revealed a potentially increased risk.

VTEs are a serious concern with a significant morbidity and mortality. The risk of VTEs is associated with the hypercoagulability related to IBD. The specific clotting mechanism have been attributed to a range of factors including thrombocytosis^[17], increased levels of clotting factors V/VIII/fibrinogen^[18], acquired antithrombin III deficiency^[19,20] and decreased levels of protein C and S^[21-23]. The exact mechanism, the interplay between the variable factors and whether the hypercoagulability

is a secondary phenomenon to IBD or represents an underlying pathological mechanism for IBD remain uncertain.

In 2001, the first large population-based study on risk of VTEs in IBD was reported from the Canadian Manitoba database. In a cohort of 5,529 IBD patients matched 1:10 with healthy controls from the general population, the risk of DVT and PE was significantly increased in IBD patients compared to controls (incidence rate ratio, IRR = 3.54, 95%CI: 2.9-4.3; and IRR = 3.3, 95%CI: 2.5-4.3 for DVT and PE respectively). IBD patients < 40 years of age were at particular high risk of VTEs with a six-fold increased risk (IRR = 6.02; 95%CI: 3.92-9.12). No sex or IBD subtype differences were observed^[24]. This study led to the introduction of thromboprophylaxis as the standard care for IBD patients with active inflammation admitted to hospital. A later population-based study from the United Kingdom by Grainge *et al*^[25] sought to elucidate the risk of VTEs during different stages of disease activity as they hypothesized that the more severe inflammation the greater risk of VTEs. In 13756 IBD patients, matched with 71627 non-IBD controls, the risk of developing VTEs was similar to the results from Canada with a hazard ratio (HR) of 3.4 (95%CI: 2.7-4.3). Further the study found that the risk of VTEs during a flare (defined as the period 120 d after a new corticosteroid prescription) was much more prominent with a HR of 8.4 (95%CI: 5.5-12.8). The highest relative risk of VTEs was found for IBD patients non-hospitalized during a flare with an almost 16-fold increased risk (HR = 15.8; 95%CI: 9.8-25.5). A recent meta-analysis identified 10 studies assessing the risk of VTEs in 72205 IBD patients and 891840 controls and found that the overall risk of VTEs in IBD was increased by 96% compared to the general population (RR = 1.96; 95%CI: 1.67-2.30)^[26]. No difference in risk was found between UC and CD. The meta-analysis further confirmed that the risk of VTEs was greater in studies including IBD patients in general (RR = 2.48; 95%CI: 2.04-3.00) compared to studies evaluating on hospitalized IBD patients (RR 1.47; 95%CI: 1.17-1.86). This observation is potentially due to an effect of thromboprophylactic treatment strategies for hospitalized IBD patients.

Only few studies have evaluated on mortality rates in VTE complicated IBD patients. From the Mayo Clinic, Solem *et al*^[26] reported a 22% mortality rate after a median follow-up of 1.8 years among 98 IBD patients diagnosed with VTE however, no comparison was made with post-VTE mortality rates in the general population. A large nation-wide population-based study from the United States by Nguyen and Sam^[27], including more than a hundred thousand IBD patients, revealed that the in-hospital mortality was significantly higher for IBD patients with VTE compared with non-VTE IBD patients and this was valid for both CD (17.0 *vs* 4.2 per 1000 hospitalizations, *P* < 0.0001) and UC (37.4 *vs* 9.9 per 1000 hospitalizations, *P* < 0.0001). The excess mortality associated with VTE was 2.1 fold higher for

IBD patients than non-IBD individuals with VTEs ($P < 0.0001$) thereby indicating that VTEs have a more severe prognosis in IBD patients than in non-IBD individuals.

To summarize, it appears evident that IBD is a moderate independent risk factor for the development of VTEs and that the risk is highest among IBD patients with a flare in disease, not admitted to hospital. Further, there is a significant mortality associated with VTEs in IBD patients that is even greater than in non-IBD patient with VTEs. This calls for the importance of preventative and treatment strategies of VTEs in the IBD population, especially in the light of results from a recent survey involving 591 United States physicians; only 35% would give pharmacologic VTE prophylaxis to a hospitalized patient with severe UC^[28].

ARTERIAL THROMBOEMBOLISM

In contrast to the well-established association between IBD and VTE, the risk of arterial thromboembolic events (ATE) in IBD is less elucidated in the literature. In the following, for simplicity, ATE will comprehend ischemic heart disease (IHD), cerebrovascular disease (CVD) and mesenteric ischemia.

Several circumstances could suggest that IBD patients are at increased risk of ATE. First of all IBD patients, particular CD patients are more likely to be current or past smokers. Further, some IBD-related drugs, *e.g.*, corticosteroids which increases the blood pressure and change the glucose homeostasis, and in contrary, the avoidance of aspirin-containing medications (due to potential fear of exacerbating IBD) could potentially increase the risk of ATE in IBD. Additionally, the presence of a chronic systemic inflammation in IBD, a well-known independent risk factor for atherosclerosis, assumedly augments the risk.

ISCHEMIC HEART DISEASE

Ischemic heart disease is caused by atherosclerotic plaque formation in coronary arteries and it is the most common type of heart disease and the leading cause of death in the world. Several inflammatory mediators as high C-reactive protein, and further up-stream inflammation markers such as tumor necrosis factor- α , interleukin-6 and 18 and the CD40 ligand are involved in the pathogenesis of both chronic inflammatory conditions including IBD and atherosclerosis^[17,29]. Further, studies have revealed that IBD patients, compared to non-IBD individuals, have an increased carotid intima-media thickness, a surrogate marker for IHD and have a higher risk of early onset of atherosclerosis^[6,30]. Thus, it appears biologically plausible that IBD patients carry an augmented risk of IHD compared to the general population.

In 2008, the first large study on risk of IHD in IBD patients, a population-based study from the Manitoba Database, Canada conducted by Bernstein *et al.*^[31], report-

ed a 26% increased risk (IRR = 1.26; 95%CI: 1.11-1.44) of IHD in 8060 IBD patients compared to non-IBD individuals. No difference in risk was observed between sex and subtype of IBD.

In contrary, a retrospective matched cohort study from United States by Ha *et al.*^[10] including 17487 IBD patients did not reveal any overall increased risk of IHD in either CD or UC, but in sub-analyses the risk of myocardial infarction was significantly increased in IBD women aged above 40 years (HR = 1.16; $P = 0.003$).

In a matched cohort study by Yarur *et al.*^[13] from 2011, the risk of IHD was assessed among 356 IBD patients and 712 matched controls and the authors reported a nearly 3-fold increased risk of IHD in IBD (HR = 2.85; 95%CI: 1.82-4.46). A nationwide Danish population-based cohort study of 4570820 individuals by Rungoe *et al.*^[12] reported a lower, although significant increased risk of IHD (IRR = 1.59; 95%CI: 1.50-1.69) in IBD patients compared to non-IBD individuals^[12]. Analyzing risk of IHD solely in the first three months and during the first year after IBD diagnosis revealed particularly high risk estimates (IRR = 4.57; 95%CI: 3.89-5.36 and IRR = 2.13; 95%CI: 1.91-2.38 respectively), hence also reflecting the potential role of ascertainment bias when assessing two chronic diseases (*i.e.* that hospitalization for one of the diseases increases the potential for discovery and recording of the other disease). However, analyses disregarding the first year after diagnosis and fully adjusted for comorbidity related medications revealed a persistent 22% increased risk of IHD over time (IRR = 1.22; 95%CI: 1.14-1.30). A following population-based Danish study by Kristensen *et al.*^[11] reported risk of myocardial infarction (MI) in more than 20.000 IBD patients according to disease activity. Analyses revealed an increased risk of MI in IBD patients during flare (RR = 1.49; 95%CI: 1.16-1.93) and during persistent activity (RR = 2.05; 95%CI: 1.58-2.65), whereas the risk was not increased during periods of remission (RR = 1.01; 95%CI: 0.89-1.15). In accordance with the Danish findings, a meta-analysis on risk of IHD in IBD by Singh *et al.*^[32] reported a 19% increased risk of IHD in IBD patients (OR = 1.19; 95%CI: 1.08-1.31) with the risk being higher in female gender (OR = 1.26; 95%CI: 1.18-1.35). Interestingly, another meta-analysis by Fumery *et al.*^[25], solely including observational studies on risk of IHD in IBD did not (potentially due to lack of power) reveal a statistically increased risk, although the magnitude of risk was similar (RR = 1.23; 95%CI: 0.94-1.62). The main difference between the two meta-analyses was the inclusion of a cross-sectional study by Sridhar *et al.*^[33] only in the latter meta-analysis; a study that contrary to expected found an inverse association between IHD and hospitalized IBD patients with a significant protective effect of IBD on risk of IHD (OR = 0.60; 95%CI: 0.56-0.65). With results paradoxical to the hypothesis authors explained this protective association could be caused by a direct result of Berkson's fallacy^[34], a form of selection bias that causes hospital cases and non-hospital controls in a case control study to be systemati-

cally different from one another, leading to a systematically higher exposure rate among hospital patients, and thereby distorting the risk estimate.

CEREBROVASCULAR DISEASE

Several case reports of ischemic stroke in remarkably young patients with CD has additionally led to the hypothesis of a potential association between IBD and CVE^[35-38].

Bernstein and colleagues reported a slightly increased risk of cerebrovascular disease in patients with CD (but not UC) in a population based setting (IRR = 1.32; 95%CI: 1.05-1.66), but adjustments were insufficient, lacking several important cerebrovascular risk factors, such as smoking, obesity and hypertension^[31]. A population-based case-control study from the United States evaluated on risk of ischemic stroke among 8054 CD patients matched with 161078 non-CD patients and results revealed an insignificant overall increased risk of ischemic stroke (OR = 1.10; 95%CI: 0.85-1.43)^[39]. A significant almost 3-fold increased risk of ischemic stroke was estimated in younger CD patients below 50 years of age (OR = 2.93; 95%CI: 1.44-5.89). A large United States conducted population-based matched cohort study found no overall increased risk of cerebrovascular disease in IBD patients, but stratified analyses revealed a significantly increased risk of stroke among women with IBD below the age of 40 compared to non-IBD controls (HR = 2.1, $P < 0.05$)^[10]. Only in a Danish setting an overall slightly increased risk of stroke in IBD patients has been estimated (RR = 1.15; 95%CI: 1.04-1.27)^[11] and during flares this risk was further increased (RR = 1.53; 95%CI: 1.22-1.92).

The meta-analysis by Singh *et al*^[32] reported pooled OR from five studies on cerebrovascular events in IBD and the meta-analysis revealed an adjusted 18% increased risk of CVE in IBD (OR = 1.18; 95%CI: 1.09-1.27), with a higher magnitude of risk estimates in women and patients at younger age.

INTESTINAL ISCHEMIA

The association between intestinal ischemia (including acute/chronic mesenteric ischemia and ischemic colitis) and IBD is vaguely elucidated. A population-based case-control study from the United Kingdom from 2011 studied risk factors for intestinal ischemia from the General Practice Research Database (GPRD)^[40]. Of the 71 cases of intestinal ischemia derived from the database only one patient had intestinal ischemia and IBD corresponding to an insignificant 4-fold increased risk (OR = 4.19; 95%CI: 0.46-38.43). From the Nationwide Inpatient Sample (NIS), the largest inpatient database in the United States, the risk of mesenteric ischemia was assessed among nearly 150000 discharges with a diagnosis of IBD and revealed a significant association between IBD and mesenteric ischemia (adjusted OR

= 3.4; 95%CI: 2.90-4.00) with a higher risk among UC patients (OR = 5.3; 95%CI: 4.24-6.74) than CD patients (2.58; 95%CI: 2.09-3.17). Young females with UC in the age group from 18 -39 years had the highest risk (OR = 15.48; 95%CI: 8.98-26.67). Likewise, a large cohort study^[10] reported increased risk of mesenteric ischemia in IBD patients with a HR of 11.2 compared with controls ($P < 0.0001$) and found the risk to be highest in UC patients (HR = 12.5; $P < 0.0001$) and females aged between 18-39 years (HR = 22.3; $P < 0.0001$). Although the absolute risk may be limited, mesenteric ischemia remains a very serious condition and IBD practitioners should be aware of the importance of recognizing these events.

CARDIOVASCULAR MORTALITY

Several studies have assessed the mortality rate from CVD in IBD and reports on both increased and decreased mortality rates exist^[41,42]. In a recent meta-analysis by Bewtra *et al*^[43] of cause-specific standardized mortality ratios in both population-based and inception cohort studies of IBD patients, no increased mortality from cardiovascular disease in neither UC nor CD was found (SMR_{UC} = 0.90; 95%CI: 0.80-1.02 and SMR_{CD} = 1.00; 95%CI: 0.88-1.13). Similar insignificant risk estimates of cardiovascular mortality in IBD patients was reported in the meta-analysis by Fumery and colleagues (pooled SMR = 1.03; 95%CI: 0.93-1.14)^[25]. Nevertheless, it is important to keep in mind that although cardiovascular mortality is a hard end-point and less prone to ascertainment bias it does not capture the entire spectrum of cardiovascular disease and with improving therapeutic options the mortality rate is decreasing and observational studies on the association between IBD and cardiovascular mortality often does not reach statistical significance due to the low mortality rates. In the large-scale population-based study by Kristensen *et al*^[11] with non-increased overall CV mortality among patients in remission (RR = 0.98; 95%CI: 0.89-1.09), authors were able to show increased CV mortality during flares (RR = 2.32; 95%CI: 2.01-2.68) and in patients with persistent disease activity (RR = 2.50; 95%CI: 2.14-2.92).

RISK FACTORS

The traditional risk factors for CVD are hypertension, diabetes mellitus, obesity, smoking, dyslipidemia, and physical inactivity.

A small Indian study by Sappati Biyyani *et al*^[14] aimed at evaluating the presence of traditional atherosclerotic risk factors in patients with IBD and coronary artery disease (CAD) compared to a control group (only CAD) by using the Framingham risk score. The Framingham risk score is a 10-year risk of CAD score based on the following risk factors: age, hypertension, diabetes mellitus, tobacco use and dyslipidemia. Among 42 cases and 137 controls the Framingham risk score was significantly

lower in patients with both IBD and CAD compared to controls (8.1 *vs* 10.0; $P = 0.002$). Yarur *et al*^[13] further assessed traditional and nontraditional risk factors in IBD related CAD and found that several traditional risk factors usually linked with patients' anthropometric status were less common in IBD. Kristensen *et al*^[11] made subgroup analyses stratifying IBD patients according to presence of traditional risk factors and showed a strong association between the number of risk factors and the risk of cardiovascular events. Additionally it is interesting that this study found an association between disease activity and risk of CV events, thereby supporting the hypothesis that the chronic inflammation acts as a risk factor for CVD in IBD patients. This is in accordance with another Danish study by Rungoe and colleagues stratifying risk according to use of oral corticosteroids, used as a proxy for both current and later disease activity, and the study revealed a higher risk of IHD in IBD patients with a history of oral corticosteroids compared to never users (IRR = 1.37 *vs* 1.23 respectively; $P < 0.01$)^[12].

POTENTIAL PREVENTIVE TREATMENTS

Considering chronic systemic inflammation as a potential nontraditional risk factor for CVD in IBD, it is interesting to evaluate the effect of treatments lowering the inflammatory burden on risk of CVD; despite the fact that anti-inflammatory therapy as treatment for atherosclerosis has received little attention. However, only few studies have addressed the impact of inflammation lowering drugs use in the management of IBD on risk of CVD.

In the study by Bewtra *et al*^[44] sub-analyses stratifying between users and non-users of 5-aminosalicylic (5-ASA), a drug potentially possessing aspirin like properties, revealed a significant decreased risk of IHD in IBD patients receiving 5-ASA compared to never users (IRR = 1.16 *vs* 1.36 respectively; $P = 0.02$)^[12]. Restricting analyses to long-term use of 5-ASA (defined as three or more redeemed prescriptions) further strengthened the finding of a preventive effect of 5-ASA on IHD (further decrease in IRR = of IHD to 1.08; 95%CI: 0.98-1.19). Interestingly, this observation of a preventive effect of 5-ASA on IHD was only present in IBD patients receiving oral corticosteroids which in this case was used as a proxy for disease severity. These results could indicate that only IBD patients with more severe disease or increased disease activity, are at increased risk of IHD and in this case the aspirin-like moiety of 5-ASA may have preventive properties.

As stated previously, the pro-inflammatory cytokine TNF- α plays an important role in the inflammatory process in both the intestine and in development of atherosclerosis. Accordingly, biological drugs impairing this cytokine, *e.g.*, infliximab and adalimumab, have been outlined not only as potential preventive treatments lowering the risk of CVD in IBD but also as a potential treatment for atherosclerotic disease as IHD

in the general population. The direct and indirect effects of the TNF- α cytokine on the cardiovascular system is very complex and to some extent paradoxical. It is beyond the scope of the present review to give a detailed description of the pathological effects of TNF- α , but overall TNF- α tends to have both beneficial and harmful effects on the cardiovascular system, both in *in vitro* and *in vivo* studies; suggestively caused by a TNF- α concentration-related difference in effect and activation of different receptors^[45-49]. This might also be the reason for conflicting results in studies evaluating the effect of TNF- α antagonist as a potential treatment option for atherosclerosis and IHD^[48,50].

A study by Greenberg *et al*^[51] evaluated on CV events associated with TNF- α antagonist treatment among more than 10000 patients with rheumatoid arthritis (RA) and found that TNF- α antagonists treatment was associated with a reduced risk of cardiovascular events compared to RA patients treated with traditional disease-modifying antirheumatic drugs (HR = 0.39; 95%CI: 0.19-0.82). The risk of CVD, including both IHD and CVE, in IBD patients treated with TNF- α antagonists was elucidated in a Danish population-based study including more than 50000 IBD patients. Thirty-one TNF- α antagonist-exposed patients and 2641 unexposed patients developed IHD, yielding an adjusted RR of 0.85 (95%CI: 0.59-1.24) whereas the risk of CVE associated with TNF- α antagonists was 1.42 (95%CI: 0.82-2.45)^[52]. Thus, point estimates indicate a protective effect of TNF- α antagonist on IHD but at the same time suggest TNF- α antagonists to be a risk factor for CVE, though noteworthy none of the estimates reached statistical significance. The complexity of TNF- α and the therapies targeting the cytokine demands for forthcoming intensive and thorough research in the field before any clear evaluation can be fulfilled.

A recent interest has been raised to the HMG-CoA-reductase inhibitors (statins), drugs mainly used for hyperlipidemia but comprise pleiotropic properties as pro-apoptotic, anti-angiogenic, and anti-inflammatory effects. The anti-inflammatory capacity of statins has been evaluated in IBD patients in a large retrospective study by Crockett *et al*^[53] revealing a 18% reduction in initiation of oral steroids in IBD patients (HR = 0.82; 95%CI: 0.71-0.94) and an even greater reduction for UC patients (HR = 0.75; 95%CI: 0.62, 0.91). Future studies are needed to clarify the beneficial effect of statins in IBD and whether a potential synergetic effect may develop due to the potential of both lowering the risk of atherosclerosis and the inflammation in IBD.

CONCLUSION

The association between venous thromboembolic events and IBD is well-established and may cause significant morbidity and mortality. Although antithrombotic prophylactic treatment is recommended for hospitalized IBD patients, surveys have shown that these recommendations are by far not followed in practice and greater

attention to this issue is warranted.

Regarding arterial thromboembolic diseases, it seems plausible and it is further supported by recent literature, that the risk of CVD is increased in IBD patients, particularly during flares. The elevated risk is most likely due to an increased atherosclerotic burden triggered by inflammatory mediators, such as CRP, interleukin 6, and TNF- α .

Future large, prospective longitudinal studies are needed to determine the true risk of CVD in IBD and to further characterize preventive and risk factors. It is of particular interest whether tight control of the IBD-related inflammation could lower the progression and early development of atherosclerosis in these patients.

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Role of bowel ultrasound in the management of postoperative Crohn's disease

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Abstract

The use of biological and immunosuppressive therapy in Crohn's disease (CD) changed favorably the course of the disease and is currently suggested in the prevention of clinical recurrence. Symptomatic exacerbation is a feature of the natural course of the disease. Endoscopic recurrence may occur earlier than clinical manifestations and its rate is still high ever since the first year after surgery. The severity of mucosal lesions is highly predictive of a new flare of the disease so that the early detection of recurrence warrants strong therapeutic changes or a closer monitoring of the case. Endoscopy is at present the gold-standard technique for the diagnosis and grading of recurrence severity, but is poorly accepted by patients for its invasiveness. A simple and easy repeatable examination able to detect early signs of recurrence could be useful in the follow-up as an alternative or as a backing in the choice of the right timing for endoscopy in questionable cases. The use of bowel ultrasound (B-US) in the management of CD has grown in the past twenty years. Its accuracy in the real time detection of the disease and its complications, known since the 80's, together with the non-invasiveness, low cost and wide availability of the technique have influenced the extension of its clinical use in many referral centers in Europe. The latest generation of ultrasound scanners

allows a precise and reproducible morphological assessment of the intestinal tract and the surrounding tissues and enables a complete evaluation of the disease. This review analyzes the literature history about B-US in the diagnosis of postoperative recurrence of CD and outlines the clinical implications of its use. Published works confirm a very good accuracy of B-US in the diagnosis of CD recurrence compared to endoscopy, also in the early phase. B-US shows a good correlation with Rutgeert's score grading, but does not prove significant association with C-reactive protein or CD Activity Index values. A wider use of B-US in the daily practice could allow to set a prompt diagnosis and an earlier and targeted treatment, probably sparing more invasive tests.

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Key words: Ultrasound; Endoscopy; Postoperative; Crohn's disease; Recurrence

Core tip: In the recent years, after the introduction of new drugs, prevention of recurrence is one of the emerging issues in the management of Crohn's disease because a more aggressive and earlier therapy is supposed to change the clinical course of the disease. Endoscopy, that is presently the standard reference for the diagnosis, is not well tolerated by patients. To assess pre-clinical signs of recurrence a non-invasive alternative is needed. Magnetic resonance imaging shows accurate results but with high costs and low availability. Bowel ultrasound can detect early specific signs of recurrence. Advantages, limits and clinical implications of the technique are discussed below.

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INTRODUCTION

The therapeutic management of Crohn's disease (CD) patients is an open challenge. The correct use of steroids, antibiotics, immunomodulators and biological therapies requires an appropriate timing in the decision making process. From this point of view an early diagnosis of postoperative recurrence is extremely important in order to identify patients with a more aggressive course and to address the correct therapeutic choice. Recurrence is endoscopically present in around 70% of patients at 1 year after surgery. Early endoscopic signs of recurrence have been detected even three months after surgery and the severity of mucosal lesions is highly predictive of future clinical manifestations of the disease^[1,2].

Endoscopy is at present the gold-standard for the diagnosis of recurrence but less invasive, repeatable techniques would fit better to follow the evolution of chronic disease if they showed comparable results. The use of computed tomography (CT) should be limited because of its biological invasiveness while magnetic resonance (MR) can not be carried out routinely for its substantial costs and inadequate availability.

Starting from the first reports in the 80's on the possibility of detecting inflammatory bowel diseases using ultrasounds, the role of this technique in characterizing inflammatory bowel disease (IBD) in terms of extension, activity and complications compared to radiology or endoscopy has steadily increased^[3-8].

In the last decade the continuous improvement in ultrasound technology enabled a better definition of the bowel wall morphology. The addition of color-power doppler, oral or intravenous contrast to advanced ultrasound (US) technical equipment made it possible to distinguish fibrotic from inflammatory involvement of the intestinal tract, phlegmons from abscesses and to select a portion of patients at increased surgical risk or with optimal response to new pharmacological approaches^[9-13].

Advantages and limits of the technique and the technical aspects of potential impact on clinical practice are discussed below.

LITERATURE ANALYSIS

All the studies available in literature define post-surgical US recurrence as an increased bowel wall thickness at the anastomosis level and the majority of them correlates US findings with endoscopy. Major obstacles to a correct interpretation of the literature are due to a significant heterogeneity in the studies' design (different reference standards and variability in the timing of procedures), in technical aspects (different cut-offs for bowel wall thickness, BWT) and in the use of additional technical equipment (Power Doppler, Enteral or Intravenous Contrast Agents).

Since 1986 DiCandio *et al.*^[14] described the possibility of detecting post-surgical recurrence using transabdominal US compared to contrast radiography and endoscopy. His pioneering work on 32 patients showed a good

sensitivity (82%) and an excellent specificity (100%) of the technique with an overall accuracy of 93.7%. In this study the possibility to distinguish between inflammatory and neoplastic lesions is shown through a structural study of the bowel wall, paying particular attention to the integrity of its layers^[14].

In 1998 Andreoli studied the US detection rate of CD recurrence in 47 patients who underwent terminal ileum resection for CD using endoscopy at the anastomotic site as the gold standard. Bowel US sensitivity was 81%, specificity 86% and the overall accuracy 83%. The authors suggest to perform US in case of clinical suspected recurrence, reserving ileocolonoscopy to negative or uncertain cases^[15].

In 2001 and 2004 two studies have been published on the role of ultrasonography in the detection of recurrence after conservative surgery (strictureplastic and/or miniresections)^[16,17]. Thickness and echopattern (the sequence of layers that constitute the sonographic appearance of the intestinal wall) of the diseased wall were considered before and 6 mo after surgery in patients with ileal strictures in order to understand if these characteristics and their postoperative behavior have a prognostic value. Both thickness and echopattern, in different measure, are relevant in order to reliably predict recurrence (hazards ratio 8.8 and 4.1 respectively).

A possible role of US as a predictor of endoscopic recurrence has been evaluated by Orlando *et al.*^[18] in 2006. Looking for the best calprotectin cut-off to assess recurrence, 50 resected patients were studied with US and fecal calprotectin every three months after surgery. Endoscopy was performed at one year. US sensitivity with a 5 mm BWT cut-off was 26% and specificity 90%. The best calprotectin cut-off value to predict the highest number of endoscopic recurrences was > 200 mg/L (sensitivity 63% and specificity 75%). Considering such a high specificity of US, the authors suggest that a positive ultrasound 3 mo after surgery, may be an indication to colonoscopy. In case of US negative, faecal calprotectin with a cut-off value of 200 mg/L could be a useful tool in order to decide if performing colonoscopy in asymptomatic patients.

In the study of Biancone *et al.*^[19] Bowel US was performed with oral contrast (small intestine contrast ultrasound - SICUS). Twenty-two asymptomatic patients, prospectively followed after surgery, underwent clinical controls every 3 mo and SICUS, wireless capsule endoscopy (WCE) and colonoscopy 1 year after surgery. Seventeen patients underwent all the 3 procedures. SICUS showed 100% sensitivity, 0% specificity (16 TPs, 1 FP), whereas WCE 100% sensitivity, 100% specificity (16 TPs, 1 TN). The small serie was then split in smaller subgroups. Considering only neo-terminal ileum recurrence and excluding patients in which disease was limited to the anastomosis the sensibility was 86% and specificity 33 %. In a very small subgroup (10 patients) SICUS and WCE were performed at 3, 6 and 12 mo. SICUS identified four of the nine WCE positive at month 3. At month 6, eight of the nine WCE positive were detected by SICUS. No

significant correlation between BWT and Rutgeert's score was found.

A part of a long term prospective follow up study on severity of CD recurrence after ileal resection published in 2010 by Pallotta *et al*^[20] reports on 58 CD patients scheduled to SICUS and ileocolonoscopy at 6 mo regular intervals after surgery. Ileocolonoscopy was performed within 2 wk from SICUS. Bowel wall thickness at the anastomosis site was measured and it correlated with the anastomotic recurrence degree sec. Rutgeerts. SICUS could detect extension of intramural lesions even in patients with tight anastomotic stenosis.

In 2010, Onali *et al*^[21] performed a longitudinal prospective study in 25 patients 3 years after surgery using oral contrast US and obtaining a very good correlation between SICUS and endoscopy. The correspondence of SICUS detected lesions with Rutgeert's grade was moderate and the attempt of identifying a bowel wall thickness value predictive for clinical recurrence did not reach statistical significance^[21].

Between 2006 and 2010 other four prospective studies comparing US performance with endoscopy have been published^[22-25]. In these studies sensitivity varies from 79% to 92% and specificity from 20% to 95%. In two of them oral contrast was used^[23,24]. For all of them ileocolonoscopy was the reference standard and bowel wall thickness (> 3 mm) the only pathological feature considered. In one paper Doppler findings were considered, slightly strengthening the accuracy only in moderate-severe recurrence and with no impact on recurrence detection^[25]. Bowel wall thickness was compared with Rutgeerts' score obtaining a good correlation between ultrasonographic findings and endoscopic lesions. Using a cut-off of 5 mm for bowel wall thickness mild from severe disease can be distinguished. No significant correlations between CD activity index (CAI) and SICUS were found^[24], while SICUS showed a higher sensitivity and specificity in detecting recurrence compared to CRP and CAI values^[23].

The use of intravenous contrast enhancement ultrasonography (CEUS) to emphasize B-US findings was reported by Paredes *et al*^[26] in a study on postoperative recurrence of CD. The sample size of the study is consistent (60 patients) and the interval between ileocolonoscopy and CEUS was 3 d only. The study considered bowel wall thickness (cut-off 3-5 mm recurrence present, > 5 mm moderate-severe), color doppler vascularity (subjectively graded) and CEUS. The authors quantify ultrasonographic activity, with a software processing of the difference in brightness of contrast enhancement maximum uptake and the baseline and worked out a US activity score that correlates with Rutgeert's degree of severity. B-US sensitivity rises with CEUS from 89.8% to 98% while specificity keeps 81%.

In the same year Cammarota *et al*^[27] published the largest retrospective study on the subject and investigate in particular the possible predictive role of BWT on surgical recurrence. All the patients included (196) were fol-

lowed for 114 mo on average and the rate of surgical recurrence was 20.4%. Bowel US was performed 6-15 mo after surgery; bowel wall thickness > 3 mm was predictive of surgical recurrence. Moreover the authors describe an increased percentage of surgical recurrence in higher values of BWT at 1 year after surgery^[27].

CONCLUSION

Several studies have been performed on bowel ultrasound and post-surgical recurrence in CD. Although most of them have a small sample size and different study designs, a very good correspondence between US and ileocolonoscopy is reported even in the early stages after surgery^[18,24]. Bowel wall thickness is the main US parameter in the detection of recurrence. The majority of the studies compare ultrasonographic with endoscopic findings and BWT values > 3 mm shows, except in two cases^[18,19], high percentage of sensibility and specificity (until 100% both) in identifying recurrence^[14,15,20-26]. Some studies demonstrate also a correlation between BWT values (> 5 mm) and Rutgeert's score severe disease grade^[19-24].

Few studies consider the echopattern performance before and after surgery in addition to bowel wall thickness^[16,17]. Morphological alterations of the echopattern are a relevant parameter in the follow-up of CD, and a good correspondence of different echopatterns with histologic findings has been shown^[28]. Moreover the predictive value of different echopatterns on the relative risk of surgical treatment and the normalization of the echopattern after biologic therapy have been reported^[9,13].

Despite the positive data supporting its use, this technique is not widespread and its use is substantially limited to some European countries. The main criticism raised by some authors is the supposed low reproducibility of the method.

Ultrasonography is by definition a subjective technique and its employment in the study of ileum and colon may be particularly difficult considering the scarcity of repere points, the high anatomical variability especially in post-surgical patients and the presence of gas in the bowel which implies the use of graduated pressure to display the deepest loops. On this issue (the reproducibility of B-US in the evaluation of Crohn's disease) a multicenter study has been performed which brought together gastroenterologists sonographers and radiologists from six referral centers for inflammatory bowel diseases, including our group. We found in different clinical settings of CD a good k value concerning BWT ($K = 0.72-1$) and the presence of complications ($K = 0.81-1$)^[29].

The performance of the examination, blinded, sequentially conducted by different operators, was preceded by a long theoretical comparison that led to the choice of parameters to be measured and methods of detection.

The results of this experience, combined with the well known positive characteristics of ultrasound (optimal tolerance, low invasiveness, low costs, wide availability) and the comparable accuracy values of B-US, CT and

MRI in different controlled settings of CD attest B-US as an added value in the clinical management of IBD^[30-32].

In our opinion features needed for a correct use of B-US are an adequate learning curve, a good clinical knowledge in inflammatory bowel diseases and the basics of ultrasound technique. The use of B-US should be included in pathways of clinical management at different levels in the management of inflammatory bowel diseases (screening IBS-IBD, therapy monitoring, follow up of complications, emergency, young children) because it raises an efficient clinical work up and reduces the use of more expensive and invasive tests with similar results in terms of clinical impact^[31,32].

It is conceivable that new technologies can improve the correspondence between imaging and the bowel wall morphology in intestinal inflammation. A wider confrontation among experienced operators on this and other interesting US parameters in B-US would be desirable.

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Quality of care in Crohn's disease

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delivery for patients with CD is not optimal at the present time and therefore needs improvement; Despite availability of national and international practice guidelines, there is a variation in the care provided to patients with CD; There is a need to develop well defined quality indicators which assures delivery of adequate care of the disease.

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Abstract

Crohn's disease (CD) is a chronic and progressive inflammatory disease of the intestine. Overall, healthcare delivery for patients with CD is not optimal at the present time and therefore needs improvement. There are evidences which suggest that there is a variation in the care provided to patients with CD by the inflammatory bowel disease (IBD) experts and community care providers. The delivery of healthcare for patients with CD is often complex and requires coordination between gastroenterologists/IBD specialist, gastrointestinal surgeon, radiologists and IBD nurses. In order to improve the quality of health care for patients with CD, there is need that we focus on large-scale, system-wide changes including creation of IBD comprehensive care units, provision to provide continuous care, efforts to standardize care, and education of the community practitioners.

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Key words: Inflammatory bowel disease; Quality assurance; Quality indicators; Outcome; Comprehensive care units; Quality improvement

Core tip: Crohn's disease (CD) is a progressive inflammatory disease of the intestine. Overall, healthcare

INTRODUCTION

Crohn's disease (CD) is a chronic and progressive inflammatory disease of the intestine, which occurs because of interaction between, immunological factors, environmental factors and gut microbiome^[1].

At the onset of the disease, the majority of patients with CD while have ulcerations in the intestine, the course of the disease gets complicated with patients developing strictures and fistula in the intestine^[2]. In a study including 297 patients with CD over 25 years, Louis *et al*^[3] reported a change in the behavior of the disease in 46% patients from non-stricturing, non-penetrating to either stricturing (27%) or penetrating (29%) disease in the first 10 years of follow-up. Because of the progressive nature of the disease, patients with CD are more likely to require not only repeated hospitalization but also surgical interventions^[4,5].

While majority of patients with CD generally present in third and fourth decade of their lives, approximately one fifth of them become symptomatic during childhood and nearly 5% of them even before 10 years of their age^[6]. Failure to thrive, retardation in the linear growth and defect in bone formation are the major issues in pediatric patients with active CD. Even puberty gets delayed in children patients with CD. Therefore, induction of re-

mission of the disease and maintenance of remission before the onset of the puberty is essential for children patients with CD. A good control of inflammatory activity is required to prevent or even minimize the consequences of a missed pubertal growth spurt and the maintenance of pre-pubertal levels of sex hormones. Since more than 90% of the bone mass is attained during childhood and adolescence, inflammatory diseases during this period can affect bone development and may ultimately lead to osteopenia and make them susceptible to fractures^[7].

Till a few years back, control of symptoms has been considered to be an end point of treatment of CD; over the past years however, healing of mucosal ulcerations has emerged as a major therapeutic goal for patients with CD^[8-10]. There are now evidences which suggest that healing of mucosal ulcerations with anti-inflammatory/immunomodulators or biologicals has a potential for changing the natural history of the disease and the available primitive evidences suggest that there is reduction in the rate of hospitalization and requirement for surgery in those patients who attains mucosal healing^[11].

The treatment of CD depends upon the activity (active phase, remission phase), location, extent and behaviour (inflammatory, stricturing, fistulizing) of the disease^[1]. The treatment needs to be tailored for each patient. The choice of treatment is also influenced by well-known negative prognostic predictors of CD such as young age of onset, presence of extensive disease, stricturing disease, and positive smoking history^[12].

Chronic disease management has become a significant focus for providing a quality and continuous care of these diseases in order to decrease their morbidity and mortality^[13]. Care of chronic diseases requires a continuous and optimal care including use of newly discovered with proven value diagnostic or therapeutic strategies. The question arises, are we providing a standard and quality care to patients having chronic diseases? In a landmark study from US, based on review of medical records and telephonic interviews, has shown that only 57% of patients attending the outpatients clinic regularly receive recommended standard of care for a variety of acute and chronic illnesses^[14]. This study has raised an important concern and further highlights the importance of delivering an evidence based care and preventative measures to patients with chronic diseases in order to decrease complications, hospitalizations, and death.

QUALITY OF CARE IN INFLAMMATORY BOWEL DISEASE IS SUBOPTIMAL

Since there is no definite cure for most patients with CD, the main objectives of treatment therefore include induction of remission and maintenance of remission; minimization of complications of the disease such as strictures, fistulae, osteoporosis, short-and long-term toxicities of the drugs; improvement in quality of life; decrease in number of hospitalizations and surgeries; and maintenance of linear growth in pediatric patients. The practice

of many chronic diseases is generally guided by evidence based literature and on the guidelines of both International and national societies^[12,15-17]. While there is some degree of variability amongst the guidelines, the essential components remain more or less similar, since such recommendations are based on the available evidences derived from a body of published literature. Since CD is a disease with heterogeneous characteristics, treatment is generally tailored or individualized for a particular patient^[12,15-18].

There is a variability in the treatment provided by an expert and a general practitioner especially for diseases, which are heterogeneous in their clinical behavior and where treatment options and guidelines are still emerging^[19]. A variability in the care of a particular disease provided by various physicians is regarded as an index for poor quality of care. In inflammatory bowel disease (IBD), there is evidence of a high degree of variation of care for both patients with UC and CD^[19]. In order to develop quality indicators for care, it is therefore, critical to understand the current status of care of such diseases. If current practice varies widely and is not well standardized, it calls for standardization of treatment protocols.

In a survey on the management of CD by IBD experts and community care providers, Esrailian *et al*^[19] reported that there was good agreement in the decision making of diagnostic testing between community care providers and the IBD experts. In the management decisions, there was significant disagreement between community care providers and IBD experts^[19]. While most community care providers in this study believed that 5-aminosalicylate products were appropriate across a variety of presentations of CD, IBD experts were significantly less likely to endorse 5-ASA use in patients with CD. In contrast to 5-ASA results, experts and community providers generally agreed with each other on the use of immunomodulators, infliximab and antibiotics in CD. Furthermore, the differences existed not only between community care providers and IBD experts; there was marked differences in the management decisions taken by various IBD experts, especially with the use of immunomodulators in newly diagnosed CD and perianal fistulizing CD^[19].

Another study including patients with CD and UC also suggested that patients with IBD often do not receive optimal medical therapy. The main points include suboptimal dosing of 5-ASA and immunosuppressive therapy, prolonged use of corticosteroids, underuse of immunosuppressive drugs, non-compliance to use of calcium and vitamin D, and inadequate screening for colorectal cancer^[20].

QUALITY IMPROVEMENT AND QUALITY ASSURANCE

Quality improvement (QI) and quality assurance (QA) are now becoming essential components of public services including delivery of healthcare services. While quality improvement is used to describe the process of

Table 1 Measures to provide quality care to patient with Crohn's disease

Delivery of high quality, safe and integrated clinical care for IBD patients based on multi-disciplinary team called IBD Comprehensive Care Unit
Delivery of care at the local center and if needed with rapid access to more specialized IBD care center
Patient education and support
Care for IBD patients that is patient-centered, responsive to individual needs
Regular audit of the care provided and outcomes

IBD: Inflammatory bowel disease.

implementing evidence-based interventions to bridge the disparities currently present in various healthcare systems; quality assurance is defined as planned, systematic activities that are implemented to ensure that a level of performance is attained^[21]. In any chronic disease process the three main objective of care include improvement in population health, improvement in patient's experience of care, and at the minimal cost; all three together are defined as Triple Aim of the disease^[22].

The essential building blocks for quality improvement efforts are the proper identification and implementation of effective quality indicators. These quality indicators are measurable elements of practice performance for which there is evidence or consensus that they may be applied to assess and improve the quality provided^[23-25]. The types of quality indicators have been broadly categorized as structural measures, process measures and outcome measures. Structural measures are indicators to do with the structure of the health system such as staffing, equipment, and electronic medical records. Process indicators are the processes of providing care such as investigations, treatment, and interactions with patients. Outcomes indicators assess the outcome of patients such as quality of life, patient satisfaction, prophylactic vaccines, mortality and morbidity. While improvement in all categories of indicators is desirable, process measures have garnered the majority of the attention, as they are most easily modifiable.

EFFORTS TO IMPROVE QUALITY OF CARE

Health care measures such as use of electronic medical record systems, automated entry of diagnostic and therapeutic orders, decision support tool at the point of care, and routine measurement of and reporting on quality have been shown to improve the quality of care^[14]. In 2004, with funding from the American Board of Pediatrics, a group of care providers started a "research and improvement network", focused on improving care for children and teens with CD^[26]. ImproveCareNow (ICN) network invited care providers to form collaboration to record information from all the patient visits and the care they were providing to children with IBD^[27]. With insitu-

tion of protocol based recording of care, the group observed an increase in the proportion of visits with complete disease classification, measurement of thiopurine methyltransferase (TPMT) before initiation of thiopurines, and patients receiving an initial thiopurine dose appropriate to their TPMT status. Furthermore, an increase in the proportion of patients either CD or UC having inactive disease on follow up was observed, suggesting a better care. The number of patients taking prednisolone also decreased^[28]. With the similar changes in the practice at IBD center at Cincinnati Children's Hospital Medical Center, there was an increase in the clinical remission rate from 59% to 76% ($P < 0.05$), decrease in frequency of steroid use from 17% to 10% and an increase in patients having Short Pediatric Crohn's Disease Activity Index < 15 from 60% to 77%^[29].

These preliminary studies from ICN are testimony that a large-scale pediatric IBD quality improvement network can change practice and improve the quality of care. The key measures required for the delivery of quality care to patients with CD is summarized in Table 1.

QUALITY INDICATORS FOR IBD

There is a lack of definitive guidelines on the measurement of quality indices in IBD. The American Gastroenterology Association has recommended 10 indices as a measurement of quality of care in IBD^[30] (Table 2). Similarly, the Crohn's and Colitis Foundation of America have also proposed a questionnaire for the assessment of quality of care of patients with IBD^[31,32] (Table 2).

In order to identify a set of quality indices, Calvet *et al*^[33] conducted a two-round web-based survey including an expert panel of patient representatives ($n = 4$), nurses ($n = 7$), surgeons ($n = 2$) and physicians ($n = 18$) using Delphi consensus-based approach. The expert panel selected a core set of 56 QIs (including 12 structure, 20 process and 24 outcome related). Structure and process quality indicators highlighted the need for multidisciplinary management and continuity of care. The key outcome quality indices focused on the adequate prophylaxis of disease complication and drug adverse events, the need to monitor appropriateness of treatment and the need to reinforce patient autonomy by providing adequate information and facilitating the patients' participation in their own care. The panel also suggested that there should be an IBD team and the team should be consisted of gastroenterologists, radiologists, surgeons, endoscopists, IBD nurse, and stoma management specialists.

HOW TO IMPROVE QUALITY OF CARE: A CONCEPT OF IBD COMPREHENSIVE CARE UNIT

The care of CD requires a coordinated action of a number of health care professionals such as a gastroenterologists/IBD expert, gastrointestinal surgeon, radiologist, stoma care personel and well trained nurses. All of them can

Table 2 Quality of care indicators in inflammatory bowel disease

Quality of care indicators	
10 quality of care indicators by American Gastroenterology Association	IBD: type, anatomic location and activity all assessed IBD preventive care: corticosteroid sparing therapy IBD preventive care: corticosteroid related iatrogenic injury - bone loss assessment IBD preventive care: influenza immunization IBD preventive care: pneumococcal immunization Testing for latent tuberculosis before initiating anti-TNF therapy Assessment of hepatitis B virus before initiating anti-TNF therapy Testing for <i>Clostridium difficile</i> - inpatient measure Prophylaxis for venous thromboembolism - inpatient measure IBD preventive care: tobacco user - screening and cessation intervention
CCFA top 10 quality outcome indicators of IBD	Corticosteroid use Proportion of patients with steroid-free clinical remission for a 12-mo period Proportion of patients currently taking prednisone Number of days per month and year lost from school or work because of IBD Number of days hospitalized per year because of IBD Number of emergency room visits per year for IBD Proportion of patients with malnutrition Proportion of patients with anemia Proportion of patients with normal disease-targeted health-related quality of life Proportion of patients currently taking narcotic analgesics Proportion of patients with nighttime bowel movements or leakage Proportion of patients with incontinence in the past month

IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor; TB: Tuberculosis.

form a IBD Comprehensive Care Unit (ICCU). While it is commonly accepted that ICCUs facilitate the provision of quality care to patients with IBD, a structure of ICCU is still not well defined^[33].

The cost of implementing some of these quality measures is modest suggesting that substantial improvement is possible. Individuals at all levels from senior clinicians to administrative staff should be encouraged to identify areas of potential improvement in the quality of care. In all settings, quality indicators should be seen as a team effort of the practice as a whole. One of the important features of chronic disease care is to provide continuous care, such as from clinic to home, interval reminder and also in between appointment care.

ANTAGONISTIC VIEW

While most supports the view that providing a quality care is a essential element of healthcare delivery system, a few believes that the imposition of quality measures may disrupt the art of medicine and the precious minutes at

an office visit may be lost in documentation rather than spending time in thoughtfully delivered health care^[22,34].

PROVIDING QUALITY OF CARE IN RESOURCE LIMITED COUNTRIES

Providing quality care in resource limited countries is a real challenge. The barrier to impart quality of CD care in resource limited countries may mainly be structure related such lack of optimal number of IBD experts, lack of diagnostic facilities, and affordability and non-referral of patients to tertiary care centers.

CONCLUSION

The delivery of healthcare for patients with CD is often complex and requires coordination between gastroenterologists/IBD specialist, gastrointestinal surgeon, radiologists and IBD nurses. Overall, healthcare delivery for patients with CD may not be the optimal at the present time and therefore needs improvement. There are evidences which suggests that there is a variation in the care provided by the IBD expert and general practitioner. To make substantial improvements in the quality of health care available to all patients, there is need of making large-scale, system-wide changes.

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Intestinal microbiota: The explosive mixture at the origin of inflammatory bowel disease?

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literature worldwide, with the aim of obtaining positive results in a number of IBD patient settings, and determining the appropriate timing and modality of this intervention. Recently, novel treatments for IBDs, such as fecal microbiota transplantation, when accepted by patients, have shown promising results. Controlled studies are being designed. In the near future, new therapeutic strategies can be expected, with non-pathogenic or modified food organisms that can be genetically modified to exert anti-inflammatory properties.

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Key words: Intestinal microbiota; Inflammatory bowel diseases; Probiotics; Prebiotics; Symbiotics

Abstract

Inflammatory bowel diseases (IBDs), namely Crohn's disease and ulcerative colitis, are lifelong chronic disorders arising from interactions among genetic, immunological and environmental factors. Although the origin of IBDs is closely linked to immune response alterations, which governs most medical decision-making, recent findings suggest that gut microbiota may be involved in IBD pathogenesis. Epidemiologic evidence and several studies have shown that a dysregulation of gut microbiota (*i.e.*, dysbiosis) may trigger the onset of intestinal disorders such as IBDs. Animal and human investigations focusing on the microbiota-IBD relationship have suggested an altered balance of the intestinal microbial population in the active phase of IBD. Rigorous microbiota typing could, therefore, soon become part of a complete phenotypic analysis of IBD patients. Moreover, individual susceptibility and environmental triggers such as nutrition, medications, age or smoking could modify bacterial strains in the bowel habitat. Pharmacological manipulation of bowel microbiota is somewhat controversial. The employment of antibiotics, probiotics, prebiotics and synbiotics has been widely addressed in the

Core tip: This paper focuses on the scientific scenario regarding the potential function of gut microbiota in inflammatory bowel diseases (IBDs). Epidemiologic findings suggest that the heterogeneity and disruption of gut microbiota can be significant in modulating and addressing the immune reactions underlying IBD pathogenesis. Traditional or innovative manipulation strategies of gut microbiota may be possible future treatment options for the management of these disorders.

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INTRODUCTION

Inflammatory bowel diseases (IBDs) are lifelong chronic disorders arising from interactions among genetic, immunological and environmental factors^[1].

Technological advances have allowed novel predictive factors to be assessed, that can identify the disease at an early stage and provide an accurate diagnosis long before the onset of clinical manifestations^[2]. Recent findings suggest that, in addition to genetic and environmental factors, interactions with the gut microbiota may play a relevant role in a “perfect storm” driving the pathogenesis of IBDs^[3].

MICROBIOTA AND IBD

The human intestinal tract includes several multifaceted microbial populations with an essential function in general health. The human gut contains, in the assortment of 1000 bacterial species, 100-fold more genes than the human genome. The new high throughput sequencing technologies, the presence of 16S rRNA genes in the gut bacterial composition, as well as recent non genomic techniques, have well defined the function of gut microbiota in some human diseases^[4,5].

Although the microbiota of the colon is apparently similar in different people, there are marked variations between individuals in bacterial populations of a single species. It has been demonstrated that an increase in biodiversity requires a different metabolic homeostasis and structural stability, while a reduction, due to age, illness or antibiotics, reduce the capacity of the intestinal environment to fight infecting pathogens^[6,7]. In fact, epidemiological evidence and experimental studies have suggested that alterations in the gut microbiota (*i.e.*, dysbiosis) can be relevant in intestinal conditions such as chronic IBD^[8].

Clinical evidence confirms the role of microbiota in IBD, and an abnormal microbial composition in IBD has been amply demonstrated. The most common site of IBD is the colon, where the highest intestinal bacterial concentrations are found. Additionally, fecal stream diversion can prevent and treat Crohn's disease (CD) and pouchitis. Finally, many studies have shown that antibiotics and probiotics improve the histological, endoscopic and clinical picture^[9]. Despite this evidence-based findings, there are still some major unexplained points such as the IBD response to immunosuppressive therapy or the protective role of poor hygienic conditions, which do not appear likely to be related to the microbial state^[10].

Animal studies

It is known that the non-pathogenic microbiota controls bowel immunity, but interactions in the gut with host microbes can be bidirectional. The mucosal immune system can be affected by the pro-inflammatory potential of abnormal growth of microbiota elements, which ultimately determine or influence an inflammatory reaction and induce the possibility of development of illness. Several animal studies have shown that this interaction is possible and can induce colitis.

Studies in germ-free interleukin 10-deficient (IL-10^{-/-}) mice, that fail to acquire spontaneous colitis and immune activation, support this hypothesis^[11]. Indeed, some stud-

ies show that, regardless of the background strain of these animals, the onset and degree of spontaneous colitis depends on the composition of the enteric gut microbiota^[11,12]. Penetrance of colitis increases to nearly 100% when the immune system response is characterized by a T-helper 1 (Th1) interferon (IFN)- γ reaction^[12].

Therefore, in this model of colitis, it has been demonstrated that the disease may show different characteristics and distribution based on the intestinal bacteria present. Furthermore, in IL-10^{-/-} germ-free mice, bacterial colonization of non-pathogenic bacteria such as *Escherichia coli* (*E. coli*) or *Bilophila wadsworthia* provokes different types of colitis^[13]. In particular, *Bilophila wadsworthia* produces a low grade colitis involving the distal colon, associated with an exclusively Th1-mediated immune response. In contrast, *E. coli* leads to an early (3-wk) development of mild-to-moderate inflammation that is more severe in the cecum. In the same study, *Bacteroides vulgatus*, but not *E. coli*, provokes mucosal inflammation of the colon in HLA-B27 transgenic mice without bone marrow involvement as in transplanted CD3 transgenic mice^[13].

Finally, novel experimental evidence demonstrated that *Klebsiella* may provoke moderate pancolitis while *Bifidobacterium animalis* could cause a mild degree of inflammation in the distal colon and duodenum^[14,15].

Human studies

A few studies in humans have suggested that IBD patients have an altered balance of intestinal microbiota in the active phase. Bacterial 16S rRNA gene examination did not show relevant differences in bacterial constitution in the intestinal mucosa of CD and ulcerative colitis (UC) patients. Moreover, in UC patients, a decrease in bacterial load was observed even if it was not significant when compared to that of CD patients^[16-18].

Another interesting finding, a thinner and less sulphated mucus in patients affected by UC, has been demonstrated and may account for an increased number of bacteria colonizing the mucosa^[19,20]. Indeed, a poor mucus layer with a microbiota overgrowth could enhance the presentation of bacterial antigens to the immune system of the gut mucosa. In UC patients, the colonic surface and inflamed areas are colonized by a broad variety of bacteria. For example, in UC specimens *Clostridium histolyticum* and *lituseburensense* accounted for 21% of the microbiota. *Enterobacteriaceae* such as *Escherichia* and *Klebsiella* have also been considered to be implicated in the pathogenetic mechanism of UC. Indeed, their aptitude to adhere to enterocytes, allowing them to penetrate the mucosal layer and deliver enterotoxins, might account for this hypothesis^[21,22].

Genetics in IBD pathogenesis

The interaction between genetic factors, and a dysregulated response of the immune system to bacterial antigens are still strongly supported hypotheses in the pathogenesis of IBD. Indeed, genome-wide association studies (GWAS) showed that several genes were associated with

IBD susceptibility^[23]. These genes, risk factors for CD and UC, encode for proteins that may regulate the microbiota (NOD2/CARD15) or may control host responses (IL-12-IL23R pathway or autophagy)^[24,25], and constitute a barrier function notably for UC^[26].

One of these proteins, NOD2, may be crucial for distinguishing between non-pathogenic and pathogenic organisms; indeed, it initiates signal transduction thus promoting NFκB translocation into the nucleus, where the expression of specific genes determines the response of primary and adaptive immune mechanisms^[27-29].

The multifunctional genetic linkage of NOD2/CARD15 is demonstrated by the protein's ability to identify bacterial muramyl-dipeptide and by its impact on the homeostasis of non-pathogenic bacteria, regulatory T cells (Tregs), and viral identification by immune system^[24]. Although NOD2 homozygosity may carry a 20-fold increased risk for CD, notably in the ileal location, less than 20% of patients affected by CD are homozygous for NOD2^[30,31]. So, while these studies and GWAS have provided important details about IBD pathogenesis, investigations on the distribution of genetic variants in different populations poorly explain the large discrepancies in IBD prevalence between different geographic areas as well as the increasing incidence of IBDs in Western countries over the past 5 decades^[2].

The evidence strongly supports that IBDs are polygenetic disorders and their heterogeneity relates to the complexity of their genetic background as well as to different lifestyle and environmental factors, including variations in microbiota composition.

Environmental triggers

It is known that nutrition, medications (NSAIDs) and smoking affect the composition of the gut microbiota and it is known that changes in this multifaceted structure are contributing factors in the origin of some disorders, including IBDs.

Smoking is a relevant risk factor in CD pathogenesis^[32-36]. Indeed, it may alter the intestinal microbiota and its cessation may further modify intestinal microbial composition. Indeed, simultaneous increased *Firmicutes* and *Actinobacteria* and decreased *Proteobacteria* and *Bacteroidetes* characterize smoking cessation; in contrast, the composition of the flora in continuous smokers and non-smokers remains stable^[37].

Many studies have reported a modification of the gut microbiota composition in populations migrating from developing to developed countries^[38]. In these subjects, diet, family size, antibiotic consumption, urbanization, reduced parasitism, and a reduction in exposure to childhood infections, such as hepatitis A and *Helicobacter pylori*, are associated with changes in the microbiota.

Neonates show a sterile or, at least, a very low microbial load in the intestine^[39]. Bacterial strains colonize the infant bowel after delivery according to various factors, such as method of delivery, breast- or bottle-feeding, and antibiotic administration^[40]. There is early colonization of

Lactobacillus and *Prevotella* after vaginal delivery and greater colonization of *Firmicutes* in neonates delivered by cesarean section, that predisposes to a greater susceptibility to some pathogens and a higher risk of atopic disease^[41,42]. Therefore, growth from newborn to early childhood and finally adulthood is associated with changes in the gut microbiota, featuring a reduction in *Lactobacillus* and *Bifidobacteria* and an increase in *Firmicutes*, *Clostridia* and *Bacteroides* species, that may lead to a high risk of allergic and immunological diseases^[43]. This raises the hypothesis that a decreased biodiversity within non-pathogenic microbiota, with an altered immunity maturation, could negatively influence the immune recognition and activation, and thereby confer a risk for developing IBD in adulthood^[38].

Regarding the impact of a high-fat dietary intake on the non-pathogenic microbiota, it has been demonstrated that it can radically remodel the intestinal microbiota^[44,45]. Moreover, there is evidence that non-absorbed carbohydrates (inulin and fructooligosaccharides) promote the growth of beneficial species, supplying a substrate for the production of short-chain fatty acids (SCFAs)^[46].

Recently, novel studies have focused on the role of NSAIDs in inducing and maintaining mucosal damage, thus contributing to the genesis of IBD. In particular, several studies demonstrated that NSAIDs were able to cause injury by means of microbiota modulation^[47]. NSAIDs, indeed, can promote the overexpression of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IFN-γ through changes in the microbiota^[48], and further allow bacterial translocation through the intestinal barrier. This hypothesis is confirmed by evidence that the levels of such proinflammatory cytokines are significantly increased in IBD patients.

Microbiota and IBDs: Comments on the literature

There can be no doubt, in view of all the experimental data, that the microbiota can be considered a key factor in the origin of IBDs and not a bystander. Studies performed on animal models provide strong evidence for a primary role played by microbiota in IBDs but human studies do not fully support this pathogenic hypothesis owing to the lack of sufficient scientific proof. For instance, it is well-known that, in CD, the entire alimentary tract from the oral cavity to the anus may be involved, but no data from human studies are available on this topic. Conversely, animal studies have demonstrated that the microbiota composition may influence the onset of IBDs in a selected part of the digestive system. El Aidy *et al.*^[49] investigated the responses of the jejunal mucosa to bacterial colonization in germ-free mice, showing a consequent shift to anaerobic metabolism, a condition that may strongly influence mucosal oxygenation in IBD. Moreover, in an experimental model of small bowel CD, a single strain of *E. coli* (LF82) has been demonstrated to stimulate the production of proinflammatory cytokines, an effect that was counteracted by lactoferrin, another microbial product^[50].

There has been much discussion as to whether infec-

Table 1 Antibiotic therapy in inflammatory bowel diseases

Ref.	Year	Antibiotics	Duration	Result
Crohn's disease-primary therapy				
Ursing <i>et al</i> ^[53]	1982	Metronidazole 800 mg/d	16 wk	No difference from sulfasalazine
Sutherland <i>et al</i> ^[54]	1991	Metronidazole 10 or 20 mg/kg	16 wk	Superior to placebo (↓ CDAL), no difference in remission
Colombel <i>et al</i> ^[55]	1999	Ciprofloxacin 500 mg 2 × d	6 wk	No difference from mesalamine
Arnold <i>et al</i> ^[56]	2002	Ciprofloxacin 500 mg 2 × d	6 mo	Superior to placebo (CDAL)
Prantera <i>et al</i> ^[57]	1996	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 4 × d	12 wk	No difference from prednisolone
Greenbloom <i>et al</i> ^[58]	1998	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 3 × d	10 wk	Uncontrolled, 68% remission
Leiper <i>et al</i> ^[59]	2000	Clarithromycin 250 mg 2 × d	4 wk	Uncontrolled, 64% response, 48% remission
Steinhart <i>et al</i> ^[60]	2002	Ciprofloxacin 500 mg 2 × d + metronidazole 250 mg 3 × d	8 wk	No improvement over budesonide alone (33% vs 38% remission)
Crohn's disease-prevention of postsurgical relapse				
Rutgeerts <i>et al</i> ^[61]	1995	Metronidazole 20 mg/kg	12 wk	↓ clinical relapse 1 yr vs placebo
Rutgeerts <i>et al</i> ^[62]	2005	Ornidazole 1 g/d	52 wk	↓ severe endoscopic relapse vs placebo
Ulcerative colitis-primary therapy				
Turunen <i>et al</i> ^[63]	1999	Cipro 500 mg 2 × d	6 mo	Superior to placebo
Mantzaris <i>et al</i> ^[64]	1997	Cipro 500 mg 2 × d	6 mo	No benefit vs placebo
Casellas <i>et al</i> ^[65]	1998	Amoxicillin 1 g/ Clavulanic acid 250 mg	5 d	↓ mucosal IL-8 and eicosanoids vs placebo
Turner <i>et al</i> ^[66]	2014	metronidazole, amoxicillin, doxycycline (Paediatrics)		Remission (46.6%)
Pouchitis				
Shen <i>et al</i> ^[67]	2001	Metronidazole 20 mg/kg or Cipro 500 mg 2 × d	6 mo	Both effective, Cipro > metronidazole
Gionchetti <i>et al</i> ^[68]	2000	Cipro 500 mg 2 × d and Rifaximin 1 g 2 × d	5 d	89% response, 33% remission, uncontrolled

tious factors could be a trigger for IBD. No evidence is available from human studies, but animal models offer interesting insights. Couturier-Maillard *et al*^[51] demonstrated that microbiota transplantation from healthy wild-type mice may reduce the IBD risk in Nod2-deficient mice and lead to long-term alterations in the gut microbiota. On the other hand, disease risk was promoted in wild-type mice that were recolonized with dysbiotic fecal microbiota from NOD2-deficient mice. In conclusion, animal models must be seen only as a starting point for microbiota investigation in humans, and the main lesson that we can deduce is that an imbalance of bacterial species is one of the main reasons that can explain the different types of colitis induced by the effect of different bacteria.

PHARMACOLOGICAL MANIPULATION OF MICROBIOTA IN IBDs

Antibiotics

Antibiotics are known to have an important role in the management of septic complications of IBD, *e.g.*, intra-abdominal and perianal abscesses and fistulae of CD, superinfections, and post-surgical wound infections. Nonetheless, treatment with antibiotics for active luminal CD and UC is not widely accepted as a first-line choice. Although the use of antibiotics against pathogenic bacteria is proven and based on reliable evidence of experimental enterocolitis and IBD, there are some clinical trials that do not sufficiently support the efficacy of these drugs in patients affected by IBD^[52].

The most representative published studies are summarized in Table 1^[53-68]. Metronidazole, ciprofloxacin, or the contemporary use of these agents are useful in Crohn's colitis, ileocolitis and pouchitis, but not in disease confined to the ileum. They are recommended for pouchitis in the European Crohn's and Colitis Organisation statements, which also indicate that ciprofloxacin appears to have fewer adverse effects (statements 8C, 8D)^[69].

Probiotics

Probiotics are viable microorganisms that have been cultured from foods, in particular milk. Various species and bacterial strains that have been used in IBD clinical trials, are believed to have a potential beneficial role. The most evaluated probiotics are *E. coli* Nissle^[70], VSL#3 mixture (four strains of *Lactobacilli*, three strains of *Bifidobacteria*, and one strain of *Streptococcus salivarius thermophilus*)^[68,71-73], BIO-THREE mixture (*S. faecalis*, *C. butyricum*, and *Bacillus mesentericus*)^[74], a mixture of *L. rhamnosus* and *L. reuteri*^[75], *L. rhamnosus* GG^[76], Yakult strains of *Bifidobacterium brevis*, *Bifidobacterium bifidum* and *L. acidophilus*^[77]. Recently, advanced genetic engineering has produced modified species that are able to produce immunosuppressive molecules such as IL-10^[78].

These studies have shown that probiotic supplementation can re-establish bacterial homeostasis in the intestine and downregulate gut inflammation that is characteristic of IBD patients, thus modulating the inflammatory/anti-inflammatory balance. A reduction in the number of microbiome elements was also found. Indeed, the administration of probiotics can normalize

Table 2 Probiotic therapy in inflammatory bowel diseases

Model	Probiotic	Effect
Trinitrobenzene sulphonic acid or dinitrobenzene sulphonic acid	<i>Bi. infantis</i>	No effect
	<i>L. acidophilus</i> , <i>L. casei</i> and <i>Bi. animalis</i>	Reduced inflammation
	VSL#3	No effect
	<i>Lactobacillus</i> GG	No effect
	<i>L. plantarum</i> 299	No effect
Iodoacetamide	VSL#3 (DNA, subcutaneously)	Reduced inflammation
	VSL#3	Reduced inflammation
Acetic acid	<i>Lactobacillus</i> GG	Reduced inflammation
	<i>L. rhamnosus</i> GG	No effect
	<i>L. reuteri</i> R2LC	Reduced inflammation
Dextran sodium sulphate	<i>L. reuteri</i> R2LC	Reduced inflammation
	VSL#3 (irradiated and DNA*)	Reduced inflammation
IL-10 knockout mice	<i>L. salivarius</i> 118 (subcutaneously)	Reduced inflammation
	<i>L. salivarius</i>	Reduced inflammation
	<i>Bi. infantis</i>	Reduced inflammation
	<i>L. plantarum</i> 299V	Reduced inflammation
	VSL#3	Reduced inflammation
	<i>L. salivarius</i>	Reduced inflammation
	<i>L. reuteri</i>	Reduced inflammation
	VSL#3 (DNA, subcutaneously)	Reduced inflammation
<i>E. coli</i> -induced colitis in IL-2 knockout mice	<i>B. vulgatus</i>	Reduced inflammation
<i>B. vulgatus</i> -induced colitis	<i>Lactobacillus</i> GG	Prevented recurrent colitis
	<i>L. plantarum</i> 299V	No prevention of recurrent colitis

altered intestinal microbiota in IBD patients, and increase protective species by reducing the pathogen load, positively affecting intestinal permeability, balancing local immune response, producing beneficial substances, and disintegrating pathogenic antigens in the intestinal lumen^[79].

In animal models (Table 2), *Lactobacilli* and *Bifidobacteria* reduced the severity of experimental colitis in IL-10 knockout mice^[80,81]. In another study *L. plantarum* prevented colitis onset in HLA B27 transgenic rats. This and other reports confirm the protective effects of several probiotics in selected hosts and special inflammatory conditions. Therefore, in experimental colitis induced in B27 transgenic rats, which had remission with broad-spectrum antibiotics, probiotics prevented recurrence of the colitis. However, probiotic treatment alone was unable to produce remission of the induced disease^[82].

The beneficial effect of probiotics was demonstrated in rats with colitis induced by instillation of 4% acetic acid, which causes altered intestinal permeability. In particular, after 4 d of acetic acid treatment the activity of myeloperoxidase (MPO) showed a 3-fold increase, in parallel with a 6-fold increase in mucosal permeability in the colonic samples. The use of *L. reuteri* R2LC, after acetic acid administration, reduced the morphological score,

MPO activity, mucosal permeability, and prevented the onset of colitis^[83].

In human studies, 9-mo daily use of a probiotic formula, *i.e.*, VSL#3, was effective in preventing the relapse of chronic pouchitis after remission induced by antibiotics^[68]. Another investigation replicated the same results, and, in addition, showed a decreased frequency of pouchitis when VSL#3 was given after pouch closure^[84].

In cases of mild-to-moderate active UC treated with probiotics, there was an improvement in clinical severity, a reduction in relapses, and induction of remission. Moreover, these findings were accompanied by high histological scores and increased levels of fecal butyrate and other SCFAs^[73-77].

Studies in UC patients found that the prevention of flare-ups by probiotics was associated with inactivation of NF- κ B, downregulation of TNF- α and IL-1 β , and a simultaneous increase in anti-inflammatory cytokines, such as IL-10^[85]. Few data are available about the mechanism by which probiotics could modify the composition of the resident microbiota, even though it has been hypothesized that they might increase the load of *Lactobacilli* and/or *Bifidobacteria*^[74,85].

On the other hand, clinical trials with the use of probiotics in CD, are less concordant than in UC. Malchow^[86] found that *E. coli* Nissle was more effective than placebo in preventing relapse of CD in the remission phase induced by conventional therapy, but supplementation of probiotics was found to be ineffective in prolonging remission after the administration of *L. johnsonii* LA1 following surgical resection^[87,88]. Similarly, a study of Prantera *et al*^[80] did not demonstrate any benefit by 1 year-long *Lactobacillus* GG consumption in the prevention of post-surgical clinical or endoscopic relapses in the neo-terminal ileum.

As reported above, Butterworth *et al*^[89] evaluated 12 potentially relevant studies of the efficacy of probiotics in CD, even though 11 did not fulfill the inclusion criteria. In the only study satisfying the stated criteria, patients with moderately active CD received *L. rhamnosus* GG for 6 mo without obtaining an improvement.

Prebiotics

Prebiotics are dietary supplementations, usually non-digestible glycosides, which are energy substrates for protective intestinal organisms. Lactosucrose, fructooligosaccharides, inulin, bran, psyllium, and germinated barley extracts promote *Lactobacilli* and *Bifidobacteria* growth, thus inducing SCFA production, in particular butyrate^[90-92]. Therefore, these substances are able to re-establish the optimal beneficial/pathogen bacteria ratio in IBD patients. These physiological dietary supplements increase the protective lactic acid bacilli load, with a consequent inhibition of harmful species by decreasing the luminal pH, reducing epithelial adhesion, and producing bactericidal molecules. Animal studies showed a protective effect in rat colitis models (Table 3)^[93,94]. Several small controlled studies but only a few randomized controlled

Table 3 Inflammatory bowel diseases prebiotic therapy

Model	Prebiotic	Effect
Trinitrobenzene sulphonic acid	Fructo-oligosaccharide	Reduced inflammation
	Galacto-oligosaccharide	No effect on inflammation
Dextran sodium sulphate	Fructo-oligosaccharide	No effect on inflammation
	Resistant starch	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
	Inulin	Reduced inflammation
	Germinated barley foodstuff	Reduced inflammation
IL-10 knockout mice	Lactulose	Reduced inflammation

trials (RCT) in IBD patients have been performed, fewer than the studies with probiotics.

Interestingly, Welters *et al.*^[95] carried out a clinical trial in 20 patients with an ileal pouch-anal anastomosis who consumed 24 g of inulin or placebo daily for 3 wk. The pH, short chain fatty acids, microflora, and bile acids were determined in the stools, while the inflammatory status was evaluated by clinical, endoscopic and histological parameters. It was proven that the treatment enhanced butyrate levels, reduced pH, and reduced the number of *Bacteroides fragilis* as well as fecal concentrations of secondary bile acids. These findings were accompanied by a reduction in inflammation in the ileal reservoir mucosa.

In another open-label study, 10 patients with active ileocolonic CD were enrolled to receive a daily 15 g dose of fructo-oligosaccharides (FOS) for 3 wk. The Harvey-Bradshaw index was chosen to assess the disease activity, and fluorescence *in situ* hybridization was used to measure *Bifidobacteria* in stools; flow cytometry of dissociated rectal biopsies evaluated mucosal dendritic cell, IL-10 and TLR expression. The results of this study were promising: the use of FOS resulted in a significant reduction in the Harvey Bradshaw index, and a significant increase in fecal *Bifidobacteria* concentrations. The percentage of IL-10 positive dendritic cells was increased from 30% to 53%. Moreover, an increase in the percentage of dendritic cells expressing TLR2 and TLR4 was found (from 1.7% to 36.8% and from 3.6% to 75.4%, respectively)^[96].

Symbiotics

Probiotic therapy can potentially be improved by simultaneous administering of prebiotics (non-digestible and non-absorbable carbohydrates) that enhance probiotic proliferation in the gut. This mixture is referred as a symbiotic. The main benefit of symbiotic formulation is that a prebiotic constituent could positively modulate the increase in local microbiota, which is further regulated by the probiotic component of the synbiotic formulation. In animal models, Schultz *et al.*^[97] evaluated the effect of a symbiotic preparation composed of a probiotic combination of *Lactobacilli*, *Bifidobacteria* and inulin (SIM) in HLA-B27-beta(2)-

microglobulin transgenic rats affected by severe colitis. After 4 mo of SIM treatment, the colonic disease showed histological improvement and, furthermore, there was an alteration in the microflora profile, with an increased variety, and specifically, increased growth of *Bifidobacterium animalis* compared with untreated rats.

A few well conducted studies have supported the usefulness of symbiotic supplementation. Furrir *et al.*^[98], in a double-blinded RCT, developed a symbiotic called Synergy 1, made up of a combination of a probiotic (*Bifidobacterium longum*) and a prebiotic (inulin-oligo-fructose), which provided a metabolic substrate for the *Bifidobacterium* strain, and obtained promising results in UC patients.

Fecal transplantation

A novel treatment for IBD is fecal microbiota transplantation (FMT). FMT consists of extracting gastrointestinal microbiota from a healthy donor, which is then instilled *via* an enema through a liquid stool suspension. FMT has recently gained ground as a therapy for refractory and/or recurrent *C. difficile* infection^[99-102].

In a recent systematic review conducted by Anderson *et al.*^[103], following Cochrane and PRISMA recommendations, 5320 articles on FMT in patients with IBD were identified. Seventeen articles were selected, including reports on FMT given in single cases to treat IBD, and in the management of infectious diarrhea in IBD. The 17 trials included 41 subjects followed up for 2 wk-13 years. FMT was able to produce a reduction in symptoms in most of the IBD patients, allow an interruption in IBD medication, and result in disease remission. In those patients who experienced a simultaneous *C. difficile* infection, complete eradication of the bacterium was achieved. Even though this procedure may face difficult acceptance by patients, the review describes promising results.

Despite insufficient data on FMT in IBD, this procedure is potentially an effective and safe treatment; it may be suggested for subjects who failed conventional treatments. It is necessary to perform new well-designed and randomized trials to enrich the data about FMT in IBD to: (1) evaluate safety and success rate; and (2) to standardize protocols. Without these considerations, FMT could not become a standard part of clinical therapy^[103].

CONCLUSION

Patients affected by IBDs, either UC or CD, suffer from a heterogeneous entity whose pathogenic etiology must be explored in the context of a “multihit” phenomenon that precipitates the disease through a multifactorial platform resulting from interactions among genetic, immunological and environmental triggers. Although the microbiota may well play a crucial role in the origin of IBD, up-to-date therapeutic strategies have a primary purpose of suppressing the host response, and so a significant fraction of patients fail to accomplish sustained remission.

While novel techniques in molecular biology and engineering have enabled further discoveries about the gut microbiota, the relationship between intestinal microbiota

and IBD has not yet been completely clarified. A better understanding of the role that some bacterial species play in IBD pathogenesis is essential in order to develop appropriate management strategies.

The possibility of modulating our gut flora by interventions on microbial composition and the correct timing of this operation have important implications on efforts to improve gastrointestinal health. Nevertheless, microbiology should support, but not replace, the genetics of IBD, and meticulous typing of the intestinal microflora should soon take a decisive place in its complete characterization in order to explore the relationship between genes and the environment in health and disease. Finally, future research in microbial intervention needs to be directed towards two areas: (1) improvements in strain selection with the goal of realizing new screening procedures for a better understanding of the mechanisms of action, and ensuring adequate efficacy; (2) a new therapeutic strategy with non-pathogenic organisms of alimentary origin that can be genetically modified with the aim of producing anti-inflammatory substances.

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Patterns of airway involvement in inflammatory bowel diseases

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Abstract

Extraintestinal manifestations occur commonly in inflammatory bowel diseases (IBD). Pulmonary manifestations (PM) of IBD may be divided in airway disorders, interstitial lung disorders, serositis, pulmonary vasculitis, necrobiotic nodules, drug-induced lung disease, thromboembolic lung disease and enteropulmonary fistulas. Pulmonary involvement may often be asymptomatic and detected solely on the basis of abnormal screening tests. The common embryonic origin of the intestine and the lungs from the primitive foregut, the co-existence of mucosa associated lymphoid tissue in both organs, autoimmunity, smoking and bacterial translocation from the colon to the lungs may all be involved in the pathogenesis of PM in IBD. PM are mainly detected by pulmonary function tests and high-resolution computed tomography. This review will focus on the involvement of the airways in the context of IBD, especially stenoses of the large airways, tracheo-

bronchitis, bronchiectasis, bronchitis, mucoid impaction, bronchial granulomas, bronchiolitis, bronchiolitis obliterans syndrome and the co-existence of IBD with asthma, chronic obstructive pulmonary disease, sarcoidosis and α 1-antitrypsin deficiency.

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Key words: Inflammatory bowel diseases; Airways; Bronchiolitis

Core tip: The lung is commonly involved in inflammatory bowel diseases; however, airway involvement is often overlooked. This review will help gastroenterologists recognize the involvement of the airways in the context of inflammatory bowel diseases (IBD), especially stenoses of the large airways, tracheobronchitis, bronchiectasis, bronchitis, mucoid impaction, bronchial granulomas, bronchiolitis, bronchiolitis obliterans syndrome and the co-existence of IBD with asthma, chronic obstructive pulmonary disease, sarcoidosis and α 1-antitrypsin deficiency, and appropriately manage their patients.

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INTRODUCTION

Extraintestinal manifestations (EIM) commonly occur in inflammatory bowel diseases (IBD), with a prevalence rate between 21%-41% reported in various series. Crohn's disease (CD) presents with EIM more frequently than ulcerative colitis (UC)^[1]. The most common EIM are erythema nodosum, pyoderma gangrenosum, arthritis, uveitis,

episcleritis, mouth ulcers, renal stones, thromboembolic disease and primary sclerosing cholangitis. Pulmonary involvement complicating IBD was originally considered rare (with a frequency rate < 1%), but the first case series published in 1976 assisted in better recognition, evaluation and description of IBD related respiratory disease^[2].

Pulmonary manifestations (PM) of IBD have been studied in the literature by small in size case-control studies, case reports and epidemiological population-based studies. Depending on the anatomic site involved, PM in IBD may be divided in to airway disorders, interstitial lung disorders, serositis, pulmonary vasculitis, necrobiotic nodules, drug-induced lung disease, thromboembolic lung disease and enteropulmonary fistulas. Concomitant occurrence of IBD with specific respiratory diseases [granulomatosis with polyangiitis (GPA), asthma, chronic obstructive pulmonary disease (COPD), alpha 1 antitrypsin deficiency and sarcoidosis] is not uncommon. Pulmonary involvement is often asymptomatic and may be detected solely on the basis of abnormal screening tests. This review will focus on the involvement of the airways in the context of IBD.

EPIDEMIOLOGY

The exact incidence and prevalence of PM in IBD is not known; however, airway involvement constitutes a large proportion, responsible for 40%-63% of overall respiratory incidents^[3,4]. PM in total and specifically airway involvement seem to occur more commonly in UC than in CD.

Although IBD related PM were originally considered rare, certain population-based studies have revealed significant interrelationships between the lungs and IBD. Bernstein and colleagues in a large population-based study in North America in 2005 reported that airway disorders in general (including asthma and bronchitis) were the most common extraintestinal manifestation in subjects with CD and the second most common in subjects with UC, with the prevalence of asthma in this population between 7%-8%^[5]. In another retrospective study from the opposite view, Birring reported that IBD was 4 times more prevalent among patients with airway diseases, particularly non-asthmatic patients with productive cough, than in the general population^[6].

These epidemiological studies along with the observation of concordant EIM among siblings and first degree relatives suffering from IBD led to genome-wide studies that showed certain genetic predispositions for various EIM. Hence, in CD, HLA-A2 and HLA-DR1 and in UC, haplotypes HLA-B27, HLA-B58 and HLA-B8/DR3 are all linked to IBD related skin, joint and eye disorders^[7,8]. To our knowledge, such a genetic predisposition for respiratory involvement in IBD has not been demonstrated yet.

PATHOGENESIS

Current theory for the pathogenesis of IBD postulates that in genetically susceptible individuals, environmental

triggering factors cause a local immunologically aberrant intestinal injury and repair as a response to commensal bacteria. Environmental factors implicated are smoking (for CD), stress, infection, drugs and diet. Polymorphisms in NOD2-CARD15 (caspase recruitment domain family member 15) are found in 25%-35% of patients of European descent with CD; haplotype HLA DRB1*0103 within major histocompatibility complex (MHC) is associated with susceptibility to and extensive UC. Various genetic mutations (ATG16L1, IL-23 receptor, TNF polymorphisms) confer to result in disease progression by loosening of the intestinal epithelial barrier, decreasing of microbial clearance, loss of tolerance of the mucosa to enteric microflora (*autophagy*) and immunological dysregulation. Innate and adaptive immunity recognize microbial antigens and through pattern recognition receptors [toll-like receptors (TLR), nucleotide binding domain like receptors (NLR)] initiate an abnormal T-cell expansion, in the form of Th-1 and Th-17 pathways in CD and mainly Th-2 pathway in UC, that lead to chronic mucosal inflammation and disease^[9,10].

Although significant progression is noted in the understanding of the pathogenesis of IBD, the exact mechanisms responsible for the cross-talk between bowel disease and airway disorders are not clearly elucidated. This association is important since clinical observations have repeatedly pointed out the phenomenon of a respiratory exacerbation occurring suddenly in patients with IBD after a therapeutic intervention (enterectomy) for their colon disease, with respiratory disease being completely unresponsive to this intervention^[11]. However, the common embryonic origin of the intestine and the lungs from the primitive foregut should be considered the basis for this association. Their common origin reflects their common structural features: an extensive luminal surface area, protected by a tight epithelial barrier that covers a submucosal layer of goblet cells, glands and, most importantly, lymphoid tissue responsible for homing of lymphocytes, as well as innate and adaptive immunity^[12].

Bronchus associated lymphoid tissue (BALT) and gut associated lymphoid tissue (GALT) are both parts of the mucosa associated lymphoid tissue (MALT). Lymphocytes become activated according to the inflammatory stimuli they receive and mis-homing of lymphocytes may provide an explanation for the migration of inflammation^[13,14]. Furthermore, as we described earlier, apart from immune-mediated phenomena, autoimmunity [pANCA, anti-Saccharomyces cerevisiae antibodies (ASCA) and antibodies against intestinal epithelial antigens] also contributes to the pathogenesis of IBD^[15]. It is therefore likely that based on common structures and the affinities of the lymphoid tissue, circulating immune complexes, stimuli and autoantibodies migrate from the intestine to bronchial and possibly alveolar epithelium, leading to airway inflammation and disease. This shift of inflammation may become more dramatic when the colon is removed after colectomy. In line with this, Adenis and colleagues in a scintigraphy study demonstrated increased pulmo-

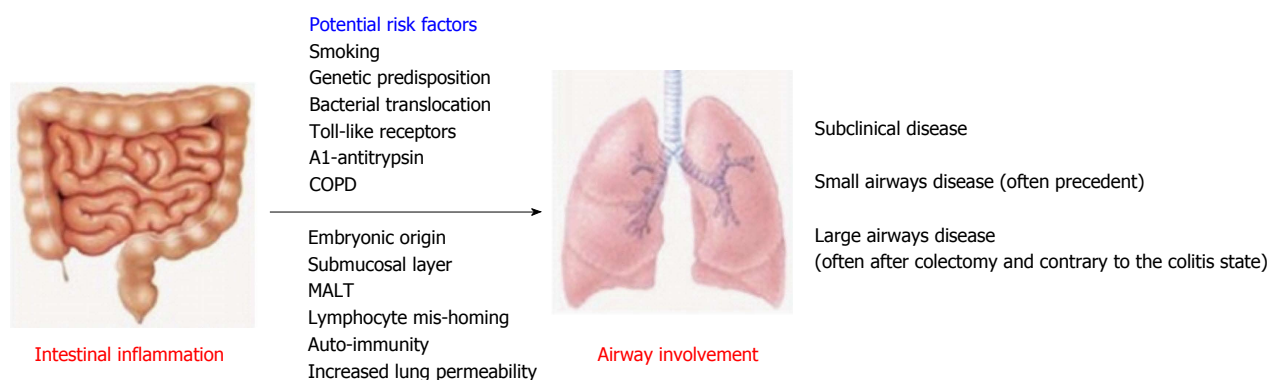


Figure 1 Possible pathogenesis of airway involvement and disease in the event of inflammatory bowel diseases. COPD: Chronic obstructive pulmonary disease; MALT: Mucosa associated lymphoid tissue.

nary permeability in patients with CD^[16].

Certain contributing factors to such presumed pathogenesis may be proposed. Smoking is a well-known risk factor both for airway diseases and CD^[17]. Bacterial translocation occurring in IBD may well affect the lung microbiome and confer to result in airway disorders since we already know that abnormal microbiome of the lungs carries implications in the pathogenesis of COPD^[18]. Common TLR molecules (TLRs 2 and 4) participate in the pathogenesis of both COPD and IBD^[19,20]. Lastly, matrix metalloproteinases (MMP) and anti-proteases like alpha-1 antitrypsin, well known as a cause of pulmonary emphysema, have been increasingly studied in the pathogenesis of IBD where their expression and balance seems to be disrupted, offering another potential link between the two systems^[21]. A proposed schema for the pathogenesis of IBD related airway diseases is shown in Figure 1.

PATHOLOGY

As described earlier in this article, in the context of IBD, pathology examination may reveal abnormalities in different lung compartments, namely the airways, the interstitial tissue, the pleura, the parenchyma and the vessels. Interstitial pneumonitis and drug related eosinophilic pneumonia have been described; however, most intriguing for the pathologist is the differentiation between CD related pulmonary involvement and GPA in the case of pulmonary nodules, particularly since CD and GPA may coincide, as shown in several reports^[22]. In this review, we shall focus on subclinical disease and airway pathology findings.

Bronchoalveolar lavage (BAL) studies have shown that chronic inflammation is common in the bronchi and alveoli of patients with CD. Wallaert *et al.*^[23] reported that 61% of asymptomatic patients with CD exhibit BAL features of an overt lymphocytic alveolitis. The clinical significance of this phenomenon, which provides further evidence for a systemic immunological manifestation of IBD to the lungs, is unknown. In our opinion, this alveolitis will not necessarily progress to clinical stage disease.

Small case series have described all types of biopsy

proven bronchiolitis, documented with wedge and transbronchial biopsies mostly but also with open lung biopsies. Granulomatous bronchiolitis is more common; acute bronchiolitis with peribronchiolar inflammation, concentric small airways fibrosis, constrictive bronchiolitis and diffuse panbronchiolitis are also reported^[4]. It should be highlighted that in the larger series by Casey and colleagues, bronchiolitides usually present before or concurrently with bowel disease, unlike other respiratory disorders that commonly follow bowel disease by a considerable time^[24].

SCREENING FOR AIRWAY DISEASES

Airway disease, either latent and subclinical or clinically active, should be recognized for several reasons: (1) airway disease may complicate and follow a course independent of the course of the primary IBD; (2) IBD related airway diseases necessitate appropriate treatment and follow-up; (3) certain pulmonary function tests (PFT) may have a role as potential markers of disease activity; and (4) screening for respiratory disease may add to the recognition of concurrent diseases such as asthma and sarcoidosis. Screening for airway disease in the context of IBD may include medical history and clinical examination, PFT and radiological examinations.

Symptoms - clinical examination

Patients examined in an IBD clinic should be regularly asked about respiratory symptoms as nearly half of them report at least one symptom which they may attribute to anything but their primary disease. The most common symptoms reported are cough, sputum production, breathlessness mainly while exercising and wheezing. Stridor and hoarseness may develop in cases of upper airway narrowing.

Camus and colleagues reported that respiratory symptoms follow IBD presentation by months or even decades in nearly 85% of patients. In 10%-15%, respiratory symptoms precede and rarely (5%-10%) coincide with inflammatory bowel disease^[3]. Respiratory symptom prevalence ranges from 25.6% in a recent study of 30 UC and 9 CD patients to 50% in an older study of 11

CD and 19 UC patients^[25,26]. In a case control study of 64 IBD patients compared to 1346 controls, after adjustment for age, sex and smoking status, IBD patients were more likely to report shortness of breath and sputum production and to a lesser degree, cough [odds ratio (OR) respectively 3.4, 2.5, 1.8], highlighting the importance of relative clinical awareness^[27].

Moreover, Higenbottam *et al.*^[28] described a respiratory exacerbation with cough and shortness of breath several years after colectomy with the primary IBD in remission. This finding has been consistently described by other authors and several case reports. Thus, respiratory symptoms may occur independently of the course and activity of the bowel disease and are not responsive in parallel to its surgical treatment; on the contrary, pulmonary disease may be exacerbated. Most of these cases are reported with ulcerative colitis.

When respiratory symptoms occur acutely, the differential diagnosis includes pulmonary embolism, infectious pneumonia and drug toxicity. When respiratory disease follows an indolent course, airway disorders are more likely. Prompt evaluation in the latter scenario should include detailed medical history (with an emphasis on smoking habit, history of asthma and medication used), pulmonary function tests and radiological exams, including high resolution computed tomography scan (HRCT) of the chest with expiratory maneuvers. Bronchoscopy, as we will discuss further, is mandatory when there is evidence of upper airway involvement.

PFT

Symptomatic IBD patients may have normal PFT; this is apparently due to the anatomic site and the recruiting capacity of the airway system (bigger for small airways, smaller for large airways). On the other hand, up to two thirds of asymptomatic IBD patients have been found to demonstrate PFT abnormalities in recent studies, a surprising finding in light of the past reports of infrequent pulmonary involvement in IBD.

A prospective study of 40 IBD patients reported a 55% frequency of abnormal PFT in active IBD, equally distributed between UC and CD. This finding fell to 17.5% when IBD went into remission^[29]. Herrlinger *et al.*^[30] reported 39% frequency of abnormal PFT in CD patients and 45% in UC, all asymptomatic, with values more affected during IBD activity but persisting in remission. Yilmaz *et al.*^[25] found abnormal PFT in 56% of IBD patients, with results directly affected by disease activity in UC cases. Another case-control study of 23 UC and 13 CD patients demonstrated abnormal PFT in 58% [75% of total events included low diffusing capacity for carbon monoxide (DLCO)], 81% of these patients with active IBD^[31]. Lastly, in the larger study of 83 UC and 12 CD patients, Desai *et al.*^[32] reported abnormal PFT in 28.5% and low DLCO in 18%. Most of these studies included a mixed CD and UC population. UC patients, however, usually outnumbered CD patients, perhaps making PFT abnormalities seem more frequent in UC.

Decreased forced expiratory volume in the 1st second (FEV1), decreased FEV1/forced vital capacity (FVC) ratio, increased residual volume (RV)/total lung capacity (TLC) ratio, low forced expiratory flow (FEF₂₅₋₇₅) and, more importantly, decreased DLCO are the parameters noted to be abnormal in IBD patients in the existing literature. While FEV1 and FEV1/FVC ratio have been found to be normal in certain older studies, other studies and recent data suggest mild functional compromise^[33,34]. Results should be interpreted with caution since different criteria have been used to define abnormal and certain studies included smoking patients or an inappropriate control population. In current studies, when an obstructive pattern has been noted, it demonstrates only partial reversibility which helps to differentiate it from asthma. RV/TLC ratio elevation has been demonstrated to correlate with bowel disease activity in several studies^[35]. Tzanakis *et al.*^[36] have thoroughly described small airways disease, particularly in the event of active UC or CD.

The most consistent finding is a reduction in DLCO that commonly correlates with disease activity. In a large study of 47 CD and 85 UC patients, decreased DLCO was found in 19% and 17.6% respectively, with values being worse when the primary disease was active^[37]. In another major study, DLCO was abnormal in 53% of inactive UC and in 81% of active UC patients; DLCO also correlated with pathological intestinal disease activity grading^[38]. In children with IBD where PFT abnormalities are rather rare, reduced DLCO was the only finding in 53% of 26 children with active CD^[39]. In conclusion, PFT and DLCO abnormalities in asymptomatic IBD are frequent but particularly so in active UC. It is believed that, as a systemic inflammatory disease, IBD affects the lung, creating a mild pulmonary inflammation corresponding to bowel inflammation. It is currently unknown, however, whether DLCO could serve as a disease activity marker.

Bronchial hyperresponsiveness (BHR) seems to occur more frequently in IBD than in a control population. This was demonstrated in 71% of children with CD and in 41% non-atopic IBD patients^[40,41]. In line with this, airway eosinophilia and deranged induced sputum have also been reported in IBD^[42]. These features may be associated with concomitant asthma or be subclinical and attributed solely to the underlying IBD. Thus, the etiology of BHR IBD may be two-fold: atopic and secondary to intestinal mucosal inflammation via the increased lung permeability observed in IBD^[16].

It is currently unclear if asymptomatic patients with PFT abnormalities will progress to clinical respiratory disease and if so what defines this progression. Until more knowledge is acquired, PFT abnormalities should dictate closer follow-up of these patients.

Radiology - high resolution computed tomography

Studies show that HRCT in patients with IBD often demonstrates abnormal findings. The most common findings include bronchial wall thickening and bronchi-

Table 1 Classification of airway diseases secondary to inflammatory bowel diseases^[1,3,4]

Site of involvement	Manifestations	Percent of total PM
Upper extrathoracic and intrathoracic airways (larynx/glottis, trachea, mainstem bronchi)	Stenoses, tracheobronchitis, acute respiratory failure	7%-8%
Large airways	Bronchiectasis	23%-26%
	Simple chronic bronchitis without suppuration	10%-20%
	Mucoid impaction	
	Bronchial granulomas	
	Suppurative bronchitis	3%-8%
Small airways	Granulomatous bronchiolitis	3%-10%
	Acute bronchiolitis	
	Diffuse panbronchiolitis	
	Bronchiolitis obliterans syndrome	
Concomitant diseases involving the airways	Asthma	
	Chronic obstructive pulmonary disease	
	Sarcoidosis	
	A1 antitrypsin deficiency	

PM: Pulmonary manifestations.

ectasis, cysts, emphysema, ground glass opacities and reticulonodular opacities. Centrilobular nodules, “tree-in-bud” opacities, air trapping and a mosaic pattern in expiratory scans all constitute a bronchiolitis radiological appearance. The prevalence ranges from 22% to 89% and radiological findings may be independent of symptoms, PFT results and primary disease intestinal activity^[32]. This is because these findings may not be solely attributed to primary IBD related pulmonary disease. Alternative diagnoses include COPD, smoking related bronchiolitis, smoking related interstitial lung abnormalities, drug toxicities, thromboembolic disease and infections.

AIRWAY DISEASES IN IBD

Airway inflammation and disease are the most prevalent and distinctive pattern of respiratory involvement in IBD and accounts for 40%-63% of the total of clinically significant pulmonary complaints. Airway diseases (AD) usually follow IBD presentation by many years or even decades and the opposite presentation is the exception; when AD occur, IBD is rather inactive. If left untreated, AD can lead to irreversible stenoses in the airway. The clinical manifestations depend on the exact anatomic site involved. A classification for AD is shown in Table 1.

Upper airways

Upper airway disease (UAD) in IBD is a rare entity that may involve pharynx, larynx, trachea and mainstem bronchi. A total of 24 cases have been reported in the literature. Exudative lesions affect the bronchial mucosa and may cause subglottic stenosis and tracheobronchitis. UAD has been described in both UC and CD, with UC

being predominant. Usually, it occurs years after diagnosis of the bowel disease, with IBD stable or in remission. UAD can occur after colectomy, with the time interval being as short as 30 d.

UAD may present with hoarseness, stridor and severe respiratory distress or just with cough, phlegm and shortness of breath^[43,44]. Physical examination may reveal wheezing during inspiration, expiration or both. Because the clinical presentation may mimic infection or asthma, a certain degree of clinical suspicion is required to suspect an insult to the upper airway as a consequence of IBD. PFT and radiology are helpful in the diagnosis. A flow-volume loop demonstrates variable extrathoracic obstruction or fixed upper airway obstruction with plateau at both phases of respiration. While chest radiography has subtle findings, a CT scan of the chest may show circumferential or nodular narrowing of the trachea and bronchi. Bronchoscopy is the diagnostic procedure of choice^[45]. Mucosal inflammation with exuberant pseudotumoral lesions, deformities, whitish lesions and narrowing of the lumen have all been described. Histology shows neutrophilic inflammation, granulation tissue, ulcerations, squamous cell metaplasia and plasma cell submucosal infiltrates. Noncaseating bronchial granulomas have also been reported in CD.

The differential diagnosis of IBD related UAD includes sarcoidosis, tuberculosis, amyloidosis and GPA^[46]. The clinical presentation may be confusing if there is no bowel disease activity or other extraintestinal manifestations of IBD or if there is anti-neutrophil cytoplasmic antibodies (ANCA) positivity. ANCA are found to be positive in 50%-90% of UC and 10%-20% of CD patients but usually are neither cytoplasmic nor perinuclear in location^[47]. The ANCA type specificity and histology of the airway lesions may help differentiate IBD-related UAD from GPA. Interestingly, isolated ANCA positivity without vasculitis has been associated with isolated subglottic stenosis in one study^[48].

Empirical data suggest initial treatment with systemically administered high doses of corticosteroids (prednisone 1 mg/kg of lean body weight administered orally or methylprednisolone 60-80 mg intravenously per day); these suggestions are drawn from case reports, however, and not from randomized controlled trials, and should therefore be critically viewed^[3]. In refractory cases, rigid bronchoscopy and interventional bronchoscopy procedures (laser beam, balloon dilation, stent placement) may be required in order to maintain an adequate airway^[49,50].

Large airways

Large airways include the bronchi from the level of lobar bronchi to the level of terminal bronchioles. Large airways are the most common anatomic site of respiratory involvement in IBD, accounting for about 50% of total PM. Bronchial disease is more common in non-smoking females in their 5th decade of life in UC, particularly when other extraintestinal manifestations are present. Bronchial disease occurs many years after IBD in 8%-85% of pa-

tients, precedes IBD in younger patients (10%-15%) and less often coincides with active IBD (5%-10%). In 79% of cases, IBD is inactive and 50% of reported bronchial disease has followed colectomy^[3,4].

The main large airway disorders are bronchiectasis (BE), chronic bronchitis (CB), suppurative bronchitis and mucoid impaction (Table 1). The classification depends on the patient's symptoms (the presence or absence of copious purulent sputum in the absence of BE classifies patient as suppurative bronchitis or CB, respectively) and HRCT features. Such features include luminal dilatation with bronchial wall thickening (definition of BE), bronchial wall thickening alone (CB) and mucoid impaction.

PFT usually reveal an obstructive pattern non-reversible to bronchodilators, or occasionally a mixed obstructive and restrictive pattern. CB diagnosis may be difficult to establish, particularly in long standing respiratory illnesses or in the presence of smoking. However, it should be noted that in most studies the reported patients were never smokers. Moreover, UC is epidemiologically connected to non-smoking individuals; in this setting, diagnosis of CB becomes more straightforward.

BE is the most important IBD related lung disorder. Typically, UC patients appear to present more commonly than CD. The bowel disease is often inactive and patients present with subacute symptoms of sputum production, cough and shortness of breath. In half of IBD-BE cases, a curative surgery (colectomy) has preceded the diagnosis of BE. In this setting, a close temporal relationship of weeks to months is well documented^[11]. Spira and colleagues reported 6 UC and 1 CD patient with BE and CB in half of them manifesting after colectomy^[51]. In another study of 14 UC and 3 CD patients, 76% developed BE, 41% shortly after enterectomy; such features are verified by other studies as well^[3,52]. The most popular explanation for this sequence implicates a shift of mediators from the resected bowel to the lung, based on their common embryonic origin. In addition, withdrawal of immune-modulatory medication like corticosteroids after IBD remission may play a role in the flare up of pulmonary disease. Autoimmunity, as postulated by other authors, may also play a role as antinuclear antibodies have been discovered in some cases^[53].

Importantly, bronchial disease is treated separately and independently from the bowel disease. As such, colectomy would aggravate rather than palliate bronchial disease. Treatment is the same as with any other cause of non-cystic fibrosis bronchiectasis. Antibiotics, bronchial toilet and bronchodilators should be offered as usual in all BE. Historically, certain authors advocate the use of corticosteroids in IBD-BE, based on case series and personal experience. These authors suggest inhaled corticosteroids should be used initially and if response is poor suggest prednisone administered orally at a dose of 0.5 mg/kg. Methylprednisolone has been also lavaged through the bronchoscope directly into the airways. Since no hard evidence supports the use of corticosteroids in IBD-BE or in bronchiectasis in general, and because of

the concern for long term treatment with corticosteroids (CS), it is the authors' opinion that CS should not be administered in primary IBD related airway diseases^[3,46,54].

Small airways

Small airways refer to the transitional airway zone from terminal bronchioles to alveolar ducts. Although larger case series attribute bronchiolitis as only 3%-10% of total IBD related pulmonary manifestations, their true involvement may be greater^[4]. Kelly and colleagues evaluated 10 patients with IBD and bronchiectasis and found that 70% of them had abnormal FEF₂₅₋₇₅, suggesting that subclinical small airways disease is more frequent^[55].

Bronchiolitis, the main form of small airway disorders, share a different clinical presentation than the clinical characteristics of upper and large airways involvement. They occur at a younger age, earlier in the disease course and in 1/3 of cases, pulmonary disease precedes intestinal disease^[4]. As such, patients with bronchiolitis who have yet to be diagnosed with IBD often undergo invasive diagnostic procedures (bronchoscopy, open lung biopsy). In contrast to other pulmonary manifestations of IBD, the bronchiolitis are equally relevant in Crohn's disease and ulcerative colitis, as opposed to rest of airway disorders that are more prevalent in UC.

Pulmonary pathology in small airway involvement has been described earlier in this article. Granulomatous bronchiolitis is the most common finding, accounting for 59% of cases, and relates to CD as a systemic granulomatous disease^[24]. PFT commonly demonstrates an obstructive pattern that may derange FEV1 or only FEF₂₅₋₇₅. DLCO is often abnormal as well^[36]. HRCT features are the same as for all bronchiolitis (as already described).

This secondary bronchiolitis may be acute or, more commonly, chronic. Chronic bronchiolitis that persists untreated significantly worsens prognosis since it may eventually progress to diffuse airway narrowing and bronchiolitis obliterans syndrome (BOS). It may also lead to the progressive formation of bronchiolectasis and bronchiectasis^[56]. This development is important because it explains physiologically the coexistence of small airways disease with larger airway involvement observed in patients with IBD. Since CS have a modest effect on small airways disease, we suggest the use of macrolides in the setting of IBD related bronchiolitis. Macrolides have shown clinical benefit in diffuse panbronchiolitis and BOS; azithromycin is shown to inhibit epithelial to mesenchymal transition and fibrosis to the small airways^[57]. Nevertheless, in cases of BOS and despite therapy, transplantation may eventually be needed.

Concomitant diseases involving the airways

Asthma: After arthritis, asthma is the most common comorbidity in both UC and CD. A large population-based epidemiological study at the University of Manitoba compared 3873 UC cases with 38674 matched controls and found a 7.88% incidence of asthma in UC (especially in males); similarly, they studied 4187 CD cases with

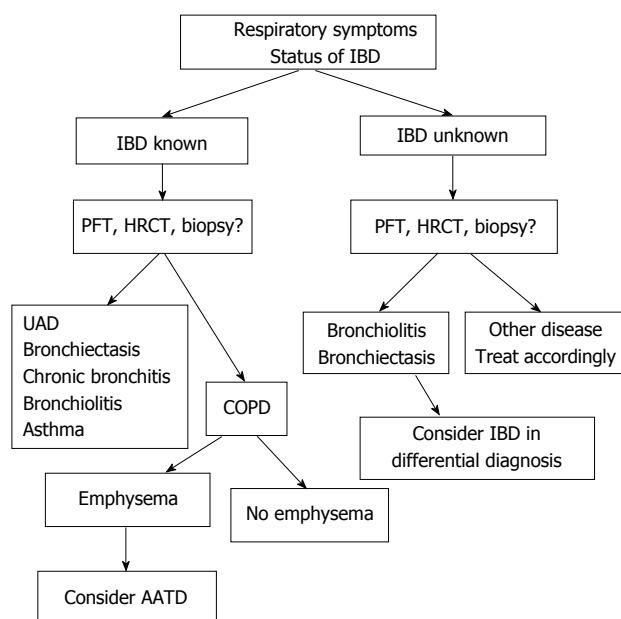


Figure 2 Proposed diagnostic algorithm for the evaluation of airway disease in inflammatory bowel diseases. IBD: Inflammatory bowel disease; PFT: Pulmonary function tests; HRCT: High resolution computed tomography; UAD: Upper airway disease; COPD: Chronic obstructive pulmonary disease; AATD: A1 antitrypsin deficiency.

41815 matched controls and found a 7.09% prevalence ratio of asthma in CD (especially in females)^[5].

We have already discussed the increased prevalence of BHR and atopy in IBD. It should be noted that when a clinical phenotype of asthma is established in a patient with IBD, appropriate treatment is mandatory since there is epidemiological evidence of increased mortality in the asthma-UC population and laboratory evidence of more severe BHR in asthma-UC patients^[58,59].

COPD: Cigarette smoke is known to protect against UC but promotes CD progression. Another population-based study investigated the relationship between COPD and IBD. Investigators found that COPD cases had a 1.83 hazard ratio (HR) for UC and a 2.72 HR for CD; this hazard extended to first degree relatives. As such, an inflammatory vulnerability in COPD patients has been postulated^[60]. A very recent study evaluating the intestinal function of COPD patients demonstrated that COPD, regarded as a systemic inflammatory disease, causes intestinal hyperpermeability and enterocyte damage leading to intestinal compromise. The latter potentially provides an explanation for this coincidence from an etiological and environmental point of view^[61].

COPD in IBD patients should be investigated, recognized and treated appropriately. COPD increases all-cause mortality and specific cause mortality in patients with CD. A meta-analysis by Duricova and colleagues reported a standardized mortality rate of 2.55 for CD-COPD subjects^[62].

Sarcoidosis: Sarcoidosis shares many common charac-

Table 2 Key messages

In a patient with IBD and respiratory symptoms, symptoms should be initially attributed to the primary disease because of significant lung-intestine interference
IBD, asthma and COPD often coincide
IBD should be always remembered in the differential diagnosis of bronchiectasis and bronchiolitis
PFT and HRCT are necessary to evaluate a symptomatic patient
IBD related airway disease does not necessarily follow the course of colitis

IBD: Inflammatory bowel disease; COPD: Chronic obstructive pulmonary disease; PFT: Pulmonary function tests; HRCT: High resolution computed tomography.

teristics with IBD, especially CD, as they both are granulomatous diseases. Sarcoidosis and IBD may coincide, as shown by case reports and population-based studies. They both share multi-organ manifestations (joints, eye, skin). Sarcoidosis and IBD seem to share a genetic overlap regarding cytoplasmic nucleotide oligomerization domains 1 and 2 and certain polymorphisms in chromosomes 1 (loci *IL-23R* and *1q.24.3*) and 15 (locus *HERC2*)^[63].

IBD, as discussed earlier in this article, may exhibit granulomatous lung disease, mainly bronchiolitis. If infections have been ruled out, it is intriguing to differentiate between an IBD pulmonary localization and concomitant sarcoidosis. Histopathological features pointing to sarcoidosis are a lymphangitic distribution of granulomas and the absence of interstitial pneumonia and chronic bronchiolitis^[24]. A clinical approach, however, is mandatory as the diagnosis of sarcoidosis apart from granuloma pathology demands compatible clinical and radiological findings.

A1 antitrypsin deficiency: Heterozygosity for the PiZ allele of $\alpha 1$ antitrypsin (AAT) deficiency (AATD) has been found to be more prevalent in patients with UC than in the general Swedish population (8.5% *vs* 4.7%)^[64]. More importantly, a recent study from the United Kingdom confirmed a higher prevalence of UC among subjects suffering from emphysema due to homozygous PiZZ allele and AATD in comparison to the general population (1.5% *vs* 0.4%)^[65]. Consequently, a blood test for AATD should be ordered when emphysema and IBD coincide in a young patient. It is unknown if AAT supplementation could be of use in the therapy of IBD.

CONCLUSION

Patients with IBD may present sometime during the course of their disease with various pulmonary incidents. The clinical approach relies upon the doctor's knowledge and judgment to attribute the patient's symptoms to the primary disease, comorbidities or to other complications after a thorough investigation. A proposed diagnostic algorithm for the evaluation of respiratory disease in IBD is shown in Figure 2. Table 2 contains the key messages

that, in our opinion, summarize the pulmonary-intestinal interrelationships in inflammatory bowel diseases.

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Use of thiopurines in inflammatory bowel disease: Safety issues

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Abstract

Thiopurines are widely used for maintenance treatment of inflammatory bowel disease. Inter-individual variability in clinical response to thiopurines may be attributed to several factors including genetic polymorphisms, severity and chronicity of disease, comorbidities, duration of administration, compliance issues and use of concomitant medication, environmental factors and clinician and patient preferences. The purpose of this review is to summarise the current evidence on thiopurine safety and toxicity, to describe adverse drug events and emphasise the significance of drug interactions, and to discuss the relative safety of thiopurine use in adults, elderly patients, children and pregnant women. Thiopurines are safe to use and well tolerated, however dose adjustment or discontinuation of treatment must be considered in cases of non-response, poor compliance or toxicity. Drug safety, clinical response to treatment and short to long term risks and benefits must be balanced throughout treatment duration for different categories of patients. Treatment

should be individualised and stratified according to patient requirements. Enzymatic testing prior to treatment commencement is advised. Surveillance with regular clinic follow-up and monitoring of laboratory markers is important. Data on long term efficacy, safety of thiopurine use and interaction with other disease modifying drugs are lacking, especially in paediatric inflammatory bowel disease. High quality, collaborative clinical research is required so as to inform clinical practice in the future.

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Key words: Thiopurines; Azathioprine; Mercaptopurine; Inflammatory bowel disease; Adult; Children; Therapeutic drug monitoring

Core tip: This review summarises the safety issues around thiopurine use in adult and paediatric inflammatory bowel disease. Adverse drug effects, toxicity and malignancy risks, interactions with concomitant medications, clinical and laboratory drug surveillance and value of pharmacogenetics in therapeutic drug monitoring are discussed.

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INTRODUCTION

Thiopurines are commonly used for maintenance of clinical remission in inflammatory bowel disease (IBD)^[1,2]. They include azathioprine, mercaptopurine (MP) and thioguanine (TG). The onset of their action may vary from 4 to 16 wk^[3-5]. Thiopurine induce apoptosis of antigen specific T cells following repetitive encounters with

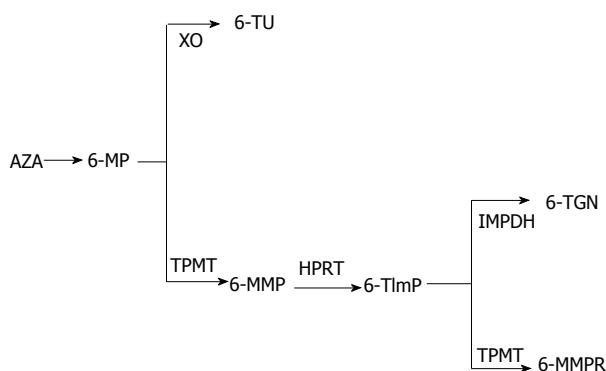


Figure 1 Metabolism of azathioprine and mercaptopurine. AZA: Azathioprine; MP: Mercaptopurine; XO: Xanthine oxidase; 6-TU: 6-thiouric acid; TPMT: Thiopurine methyltransferase; 6-MMP: 6-methylmercaptopurine; HPRT: Hypoxanthine phosphoribosyltransferase; 6-TIMP: 6-thioinosino-5' monophosphate; IMPDH: Inosine monophosphate dehydrogenase; 6-MMPR: 6-methylmercaptopurine ribonucleotides; 6-TGN: 6-thioguanine nucleotides.

the antigen over a prolonged period of time^[6]. Continued medium to long term use is recommended^[7], however there is lack of evidence about optimal treatment duration so as to sustain the therapeutic efficacy in both Crohn's disease (CD) and ulcerative colitis (UC)^[8-11].

Safety issues and risk/benefit analysis must be considered prior to and during treatment with thiopurines, therefore regular follow up for detection of poor response, dose adjustment or treatment modification is necessary. Surveillance is essential for prompt identification of adverse drug events, thiopurine toxicity, interactions with concomitant medication and loss of response over time. Factors including genetic differences, age, disease duration and severity, comorbidities and the environment may influence treatment efficacy and safety^[12-14].

MECHANISM OF ACTION OF THIOPURINES

Thiopurines are structural purine analogues which, when orally administered, are absorbed across the gastrointestinal tract epithelium and carried by the portal venous system to the liver before entering systemic circulation. Trials of intravenous thiopurine infusions have been reported^[15,16]. Azathioprine is a pro-drug with good oral bioavailability and long duration of action. In the liver, azathioprine undergoes enzymatic^[17,18] and non-enzymatic reduction in the presence of glutathione and releases MP and 6-TG. There is a paucity of evidence on the use of thioguanine^[19], but its use has been associated with severe irreversible hepatotoxicity and therefore is not currently recommended outside a clinical trial setting^[20]. Small scale retrospective cohort studies by van Asseldonk *et al*^[21] and Herrlinger *et al*^[22] advocate short term efficacy of thioguanine for maintenance of remission; however the risk of hepatotoxicity outweighs potential clinical benefits. MP is converted to thioinosine monophosphate (tIMP) by hypoxanthine guanine phosphoribosyltransferase (HGPRT); tIMP is either converted to inactive inosine-triphosphate

(ITP by ITPase) or 6TImP (6 thioinosino-5' monophosphate), by thiopurine methyltransferase (TPMT), which inhibits nucleic acid synthesis. Through this cytotoxic effect on dividing cells, thiopurines inhibit clonal proliferation during the induction phase of the adaptive immune response; as a consequence, antibody and cell mediated immune responses of the effector phase are also suppressed^[23]. 6-TG is ribosylated and phosphorylated by HGPRT and irreversibly inhibits 6-thioguanine nucleotides (6-TGN) production. Various metabolites are enzymatically produced during this process: mainly active metabolites are 6-TGN, which mediate the pharmacologic effect of thiopurines, and 6-methylmercaptopurine ribonucleotides (6-MMPR) which are formed by TPMT and principally mediate thiopurine induced hepatotoxicity (Figure 1).

The immunosuppressive effect of thiopurines has been reported to be exerted by alternative mechanisms; for instance by gene expression suppression of inflammatory genes such as α4-integrin, tumour necrosis factor (TNF) ligand superfamily member 10 and TNF receptor superfamily member 7^[24]. Other proposed in vitro mechanism by Tiede *et al*^[25] is through inhibition of GTPase Rac1 activation by azathioprine metabolite 6-thioguanine triphosphate (6-Thio-GTP) in CD4⁺ human T lymphocytes^[26].

THERAPEUTIC EFFECTIVENESS OF THIOPURINES

Recent Cochrane reviews have highlighted that azathioprine is beneficial for maintenance but not induction of remission in active CD and UC^[10,27]. Thiopurines exert indirect steroid sparing effect due to their effectiveness in sustaining prolonged clinical remission^[10,28,29], especially when used in moderate to severe IBD^[30]. Reduced rates of first laparotomy in adults with CD and positive effect in the avoidance of advanced colorectal cancer have been reported^[31,32]. No clear evidence on surgery sparing benefit has been demonstrated in a population based study which investigated the effect of immunosuppressive drugs and surgery rates over time^[33]. Thiopurine use has been shown to improve quality of life in adult patients with IBD^[34,35]. Children also tolerate thiopurines and can achieve prolonged remission^[36,37].

A meta-analysis by French *et al* investigated relapse rates following withdrawal of azathioprine in CD and reported lack of strong evidence in support of continuous thiopurine treatment beyond 18 mo for maintenance of remission in CD^[38]. Fraser *et al* in a 30 years review reported sustained efficacy for five years in adult patients with CD and UC^[8]. A randomised controlled trial by Hawthorne *et al*^[39] reported that early withdrawal of azathioprine in UC patients in clinical remission for at least six months, resulted in significantly higher relapse rate, when compared to patients on minimum of two years continuous treatment. Thiopurines are therefore safe, effective medicines with a pivotal role in the treatment of

Table 1 Reported incidence of thiopurine adverse drug effects

ADE	Incidence
GI symptoms ^[155]	8%
Hepatotoxicity ^[179,180]	3%-15%
NRH ^[51]	0.6%-1.28%
Pancreatitis ^[155]	4%
Leucopenia ^[180]	2%-15%
Myelotoxicity ^[155]	4%
Lymphoma ^[155]	0.1%
Infections ^[180]	0.3%-7.4%
Intolerance ^[57,155]	17%

ADE: Adverse drug effects; GI: Gastrointestinal; NRH: Nodular regenerative hyperplasia.

IBD^[40].

THIOPURINE ADVERSE DRUG REACTIONS: IMPLICATIONS FOR SAFETY

Adverse drug reactions have historically been divided in idiosyncratic and intrinsic (dose dependent). The first type is probably immune mediated, unpredictable and can occur within few weeks of treatment commencement. Reactions include intolerance and hypersensitivity manifestations, such as malaise, dizziness, vomiting, diarrhoea, fever, myalgia, arthralgia, rash and hypotension^[41]. Rare idiosyncratic reactions include renal impairment^[42], pneumonitis^[43-45], and pancreatitis^[46].

Thiopurine-induced liver dysfunction secondary to methylated intermediate metabolites can manifest as elevated liver enzymes, hepatitis, cholestatic jaundice or hepatic veno-occlusive disease. Liver impairment following thiopurine administration has been divided into three categories: hypersensitivity, idiosyncratic cholestatic reactions, and nodular regenerative hyperplasia (NRH). Characteristic nodules of hypertrophied hepatocytes with adjacent areas of atrophied hepatocytes arise following thiopurine induced sinusoidal endothelial injury or obliterative portal venopathy^[47,48]. Dose-dependent toxicity is possible in cases of NRH^[20,49]. The cumulative incidence of NRH in IBD patients treated with thiopurines is approximately 0.6% and 1.28% at 5 and 10 years respectively^[50]; patients on higher dose of thiopurines have increased risk of NRH^[51]. NRH however can occur in thiopurine-naïve patients with IBD (reported incidence 6%) and IBD may be an independent predisposing risk factor^[51]. Pathogenesis of NRH is obscure. Reported risk factors for NRH, other than thiopurine dose in patients with IBD, are male gender, older age, stricturing disease and small bowel resection^[50,52,53]. Mild liver impairment presenting with raised liver function tests, which represents the majority of cases, may resolve with or without dose reduction^[20].

Bone marrow toxicity manifesting as myelosuppression and/or aplasia is the most severe haematological adverse drug reaction and leads to discontinuation of treatment^[54]. Haematological toxicity presenting as leucopenia

is presumed to be avoided with reduced doses^[55], however there are studies which do not support that this type of toxicity is dose related^[56,57]. Patients with UC treated with immunomodulators have demonstrated a higher incidence of infections than patients not on thiopurines^[58], due to impaired immune response. In particular, patients with inflammatory bowel disease are at increased risk of acquiring opportunistic infections (OR = 3.1; 95%CI: 1.7-5.5)^[59], for instance cytomegalovirus (CMV) infection (especially pneumonitis or enteritis), which may cause aggravation of underlying disease and failure of immunosuppressive treatment^[60]. Pneumocystis jiroveci pneumonitis is an opportunistic infection with severe morbidity. Antibiotic prophylaxis should be considered on a case by case basis, especially in patients with advanced age, increased disease severity and extensive disease^[61]. Parasitic or other fungal infections are extremely rare in IBD^[62].

Due to thiopurine induced immunosuppression, 3-5 yearly pneumococcal and annual prophylactic influenza vaccinations with trivalent inactivated vaccine are recommended^[62]. International consensus guidance recommends varicella, tuberculosis, Hepatitis B, Hepatitis C, HIV screening and prophylactic vaccination (not BCG) prior to treatment with immunomodulator^[62,63].

The incidence of adverse drug events are summarised in Table 1.

SAFETY OF THIOPURINES IN ELDERLY PATIENTS

In the elderly population, there is good evidence of functional alterations in cells from the innate and adaptive immune systems resulting in a state of dysregulated immune function and increased susceptibility to infection^[64-66]. There are data to demonstrate that mainly bacterial infections (urinary tract infections and community acquired pneumonia), and few viral infections, such as influenza, are more prevalent and severe in the elderly patients with IBD than in younger adults^[66].

Elderly patients (> 65 years of age) on immunosuppressants are at increased risk of developing malignancies^[67], when compared to younger adults and children. Past or co-existing comorbidities, such as previous cancer, predispose them to additional malignancy risk, for example lymphoma^[68].

In elderly patients therefore, the benefit of long term (over 5 years) thiopurine use may not outweigh the risks^[69]; disease chronicity and severity may of course exert a confounding effect in observed outcomes in this population. Adverse drug events such as NRH have been more frequently reported in older age^[51].

SAFETY OF THIOPURINES IN CHILDREN

Variation in disease management is common in paediatric IBD due to lack of high quality randomised controlled trials in children. The ongoing development of service networks will accelerate collaborative standardised re-

search in children with IBD for generation of high quality evidence and improvement of care^[70].

The thiopurines have been rarely implicated in lymphoproliferative disorders in childhood IBD. The relative risk is 3-4 folds increased, however the absolute risk is very low^[71].

In paediatric IBD, early life onset of disease translates into longstanding disease activity requiring life-long medication^[72]. Paediatric-onset UC has a different phenotype than adult-onset disease with more extensive (pan colitis) and more aggressive disease course. Special consideration in the decision making process about treatment in children must be given to growth, puberty and bone density accrual. A large number of children are at risk for steroid-dependency, therefore steroid sparing strategies with early use of immunomodulators such as thiopurines are recommended in high-risk patients^[73]. On the other hand, the safety profile of immunosuppressive therapy in children stipulates a more conservative approach, with early treatment intensification applied in patients with severe or refractory disease^[12]. Punati *et al.*^[30] published a prospective multicentre observational study where early thiopurine use was associated with reduced corticosteroid exposure and possibly fewer hospitalizations per patient. Similarly, Riello *et al.*^[37], in a retrospective study of 105 children treated with thiopurines, reported that the majority of patients who were in steroid-free remission by 12 mo, remained in prolonged remission.

SAFETY OF THIOPURINES DURING PREGNANCY

Use of thiopurines is not an absolute contraindication throughout pregnancy; however data on their safety profile during pregnancy is insufficient. The human placenta is believed to act as a barrier; a human placental perfusion model has been used to demonstrate the binding of the drug to placental tissue. Maternal pharmacokinetic parameters could restrict the fetal exposure to drug metabolite^[74]. Fetal 6-TGN levels correlated with maternal 6-TGN in a prospective study of 28 pregnant women on thiopurines; maternal thiopurine metabolism was affected during pregnancy. 60% of the neonates were noted to be anaemic at birth^[75]. Casanova *et al.*^[76] conducted a retrospective multicentre study with 571 pregnant women and found no increase in adverse outcomes for pregnant women and newborns following exposure to thiopurine. There is no evidence whether IBD or medical therapy [5 aminosalicylates (5-ASA), thiopurines, corticosteroids] during pregnancy increase the risk of major congenital anomalies in the off-springs; this was recently shown in a retrospective case control study of women with IBD ($n = 1703$) and women without the disease ($n = 38481$)^[77]. Akbari *et al.*^[78] identified the risk of preterm delivery in a recent meta-analysis which reviewed the effects of thiopurines on birth outcomes of female and male patients with IBD; despite this, exposure to thiopurine at the time of conception was not associated with increased risk of

congenital abnormalities. The development and immune function of children exposed to thiopurines in utero has not been affected until the age of six years^[79].

RISK OF MALIGNANCY AND THIOPURINES: IMPLICATIONS FOR SAFETY

Thiopurines can increase the incidence of malignancies by different plausible mechanisms, such as by incorporating 'rogue' thiopurine nucleotides in the DNA or by rendering DNA highly sensitive to ultraviolet radiation, thereby promoting mutagenesis^[80-82].

Four to six fold increased risk of hematologic malignancies^[83] has been observed in patients treated with azathioprine; however no causality has been established to date. Risk increases gradually over successive years of therapy and discontinuation of thiopurine therapy reduces the risk^[84]. The absolute risk of lymphoma however is low; balancing the potential risk of lymphoma against the risk of undertreatment IBD should inform decision making in the medical management of IBD^[85].

Latent or primary opportunistic EBV infection during immunosuppressive therapy may result in post-transplant like lymphoproliferative disease or haemophagocytic lymphohistiocytosis^[86,87]; the latter has also been reported following CMV infection^[88]. 5% of EBV negative peripheral T cell lymphomas reported in IBD patients are non-Hodgkin's hepatosplenic T cell lymphomas (HSTCL) with poor prognosis^[89,90]. A systematic review on medication, therapy duration and patient age in reported cases of HSTCL concluded that most patients were male, younger than 35 years old, and had received at least 2 years of combined treatment with anti-TNF agent and thiopurine^[91] or anti-TNF monotherapy^[92].

Relative increase in non-melanoma skin cancer was shown in a large retrospective cohort study of over 50000 adult patients with IBD, conducted by Long *et al.*^[93]; thiopurine treatment of a minimum of three months has been associated with increased risk of non-melanoma skin cancers compared to controls. Melanoma skin cancer may increase by 37% according to a meta-analysis investigating the risk of melanoma in a cohort of 172837 patients with IBD; however no specific increase in risk has been associated with thiopurine treatment *per se*^[94].

Since the beginning of this century, there is a controversy in the medical literature with regards to increased risk of gastrointestinal neoplasia in patients with IBD.

A meta-analysis by Eaden *et al.* in 2001 investigated the colorectal cancer risk in UC, and reported a risk of 3% (95%CI: 2.2-3.8) at 10 years, 5.9% (95%CI: 4.3-7.4) at 20 years, and 8.7% (95%CI: 6.4-10.9) at 30 years after diagnosis. A non-significant increase in the risk of colorectal cancer (CRC) by decade of disease was also reported. In 2005, a more recent meta-analysis of population based studies by Jess *et al.* however reported a 2.4 (95%CI: 2.1-2.7) fold increase in CRC risk in patients with UC^[95].

Canavan *et al.*^[96] performed a meta-analysis to ascertain the combined relative risk of gastrointestinal malignancies and reported increased relative risk in CD. The most recent meta-analysis by Lutgens *et al.*^[97] has updated CRC risk in both ulcerative and Crohn's colitis, by investigating time trends, and identifying high-risk modifiers; it has been concluded that the risk of CRC is increased in patients with IBD but not as high as previously reported and that the risk of CRC is significantly higher in patients with longer disease duration, extensive disease, and IBD diagnosis at young age^[97].

Thiopurine treatment did not decrease the risk of colorectal neoplasia in UC ($n = 315$ patients)^[98]. Beaugerie *et al.*^[99] nevertheless conducted a large prospective cohort study with 20000 patients and found that thiopurine treatment significantly lowered the multivariate adjusted hazard ratio for colorectal neoplasia (HR = 0.28, 95%CI: 0.1-0.9; $P = 0.03$). A recent meta-analysis by Gong *et al.*^[100] which addressed the same issue concluded that thiopurines exerted a chemo prophylactic effect and a tendency of reducing advanced colorectal neoplasms in IBD, however due to the heterogeneity of included studies, the authors suggested that the results should be interpreted with caution.

Young female smokers with concomitant 5-aminosalicylic acid and thiopurine exposure^[101] are at increased risk of cervical cancer; it is still unclear whether the disease itself or whether the treatment may predispose to cervical dysplasia^[102]. Evidence to date is inconclusive; no clear increase in relative risk secondary to the use of thiopurines has been shown in the majority of relevant studies, with the exception of a population based case control study by Singh *et al.*^[103], which reported increased risk for cervical dysplasia or cancer in patients on both steroids and immunomodulators. Lees *et al.*^[104] conducted a large case control study where women with IBD were not shown to have increased rates of abnormal cervical smears unless they smoked; this finding was not affected by immunosuppressant therapy or disease phenotype. Cervical cancer surveillance and HPV vaccination is currently recommended^[102].

Treatment with immunosuppressive drugs had no major impact on the increased risk of developing new or recurrent cancer in a prospective cohort of 405 IBD patients with pre-existing history of cancer^[105]. Pasternak *et al.*^[106] conducted a retrospective cohort study and reported that azathioprine treatment in IBD was associated with marginally increased risk for lymphoma and urinary tract cancer, therefore reported an overall increased risk of malignancy with azathioprine treatment (RR = 1.41; 95%CI: 1.15-1.74) interestingly though, previous use of azathioprine or increasing cumulative received doses did not increase the cancer risk. Finally, a meta-analysis of Masunaga *et al.*^[107] investigated whether long-term administration of immunosuppressants in patients with IBD increased the risk of malignancy and concluded that treatment did not increase the overall cancer risk in patients with IBD; drugs other than thiopurines, such as cyclosporine, methotrexate, tacrolimus were included in the analysis.

CLINICAL AND BIOCHEMICAL SURVEILLANCE FOR ENHANCED DRUG SAFETY: THERAPEUTIC DRUG MONITORING

Clinical surveillance and patient follow up are required for identification of adverse drug events throughout the duration of thiopurine treatment; this can be enhanced by regular monitoring of laboratory indices, such as peripheral blood counts, pancreatic and liver function tests^[108]. There is a lack of consensus on the usefulness of active thiopurine metabolite monitoring as surrogate markers of thiopurine efficacy and toxicity. Their use is not widely recommended or adopted^[109] and largely depends on local practices and individual clinician preferences.

Higgs *et al.*^[110] published a meta-analysis about the increased risk of myelosuppression in patients with intermediate TPMT activity and concluded that in spite of controversial findings between studies, "higher 6-TGN levels were generally associated with clinical remission". Gonzalez-Lama conducted a prospective multicentre cohort study which did not support determination of TPMT activity or 6-TGN concentrations for prediction of treatment outcome; no clinically useful serum metabolites threshold value was identified for dose adjustment purpose^[111]. Poor correlation between 6-TGN levels and thiopurine dose has also been reported by other studies^[4,56,112,113], however metabolites levels may contribute to earlier and safer dose tailoring, especially in patients with poor clinical response or non-compliance^[114-117].

Measurement of active thiopurine metabolite levels in addition to regular full blood count monitoring, especially over the first 1-2 mo after treatment commencement, and regularly thereafter has been proposed^[118-120]. 6-MMPR levels and liver enzymes may be used in combination as surrogate markers of hepatotoxicity; a 6-MMPR cut-off value $> 5700 \text{ pmol}/8 \times 10^8 \text{ RBC}$ has been recommended as indication of liver dysfunction^[117,121-123]. There is currently no clear consensus on optimal frequency of blood monitoring^[124].

DRUG INTERACTIONS: IMPLICATIONS FOR THIOPURINE SAFETY PROFILE

Allopurinol

Allopurinol is known to reduce thiopurine-induced hepatotoxicity^[125] and may have a synergistic therapeutic effect to patients who preferentially produce 6-MMPR, rather than 6-TGN^[126-128]. The underlying mechanism remains enigmatic. Different pathways have been proposed: (1) allopurinol inhibition of both xanthine dehydrogenase and TPMT and promotion of 6-TGN production at the expense of 6-MMPR^[129]; (2) TPMT inhibition by allopurinol's active metabolite oxypurinol^[130]; and (3) increased HGPRT activity and subsequent 6-TGN increase^[131]. Co-administration of allopurinol has been reported to al-

low thiopurine dose reduction by up to 75%^[23]; this may enhance efficacy in azathioprine refractory patients and avoid high doses with potential dose dependent adverse events; conversely combined therapy could potentially induce toxicity in azathioprine-naïve patients due to the synergistic effect of these two medicines. Combination therapy with allopurinol is therefore currently not widely used, due to the lack of adequate high grade evidence from double blinded randomised controlled trials assessing the risk-benefit ratios of dual therapy, especially in paediatrics.

5-aminosalicylates

5-ASA have been reported to increase 6-TGN and decrease the production of 6-MMPR in two prospective adult studies^[132,133]. de Boer *et al*^[134] conducted a prospective multicentre pharmacokinetic study and reported a significant dose-dependent increase in 6-TGN levels in cases of 5-ASA co-administration. It was concluded that patients refractory to standard thiopurine therapy may benefit from the co-administration of 5-ASA. A systematic review by Andrews *et al* addressed the clinical outcomes following concomitant 5ASA and thiopurines administration; it was unclear whether combination therapy improved outcomes of disease control, drug toxicity or compliance, but concurrent therapy could decrease colorectal risk at “acceptable cost”^[135]. An increased risk of myelotoxicity has been noted in children with IBD treated with combination therapy^[136]. Nguyen *et al*^[137] in a study of 71 children with IBD, reported more frequently observed lymphopenia and elevated 6-TGN concentrations, without increase in remission rate in patients on combined treatment. A favourable clinical outcome has been described by Tajiri *et al*^[56] in paediatric UC, however a high rate (40%) of myelosuppression was noted. The clinical benefit of combination treatment therefore needs to be further researched and careful weighted against toxicity risk^[132].

Anti-tumour necrosis factor alpha agents

New disease modifying drugs such as infliximab, adalimumab have been increasingly used in adult and paediatric gastroenterology. Infliximab is the first anti-TNF alpha agent ever introduced in the treatment of IBD and therefore has been more widely researched to date. It is a partially humanised monoclonal antibody against tumour necrosis factor alpha (TNF- α), a cytokine mediator of inflammation. Historically anti-TNF alpha drugs were principally introduced as rescue medical therapy in patients with treatment refractory, severe or extensive disease^[138].

A top-down versus a “bottom-up” approach to treatment is the epicentre of attention within the international community of gastroenterologists; early introduction versus rescue use of anti-TNF agents, with or without concomitant thiopurine for prompt treatment intensification and avoidance of disease complications is the dilemma in current clinical practice^[139,140].

Combination therapy with infliximab and azathioprine

has been reported more favourable than monotherapy for induction and maintenance of steroid-free remission, and for avoidance of postoperative recurrence^[140,141]. Higher serum trough concentrations of infliximab, lower anti-infliximab antibodies and better clinical outcomes may occur more frequently in patients receiving combination therapy with azathioprine^[139,142]. Colombel *et al*^[139] specifically reported that combination may be superior to azathioprine or infliximab alone, not only for induction and maintenance of steroid free remission, but also for increased mucosal healing rates in adult patients with moderate to severe CD. The described effect was sustained at one-year follow up. The beneficial effects of combination therapy on mucosal healing, steroid-free remission and sustained increase in quality of life have been reported in both UC^[143] and CD^[144]. Sokol *et al*^[145] conducted a prospective cohort study and reported favourable six month outcomes with regards to disease activity, infliximab dose and need of other anti-TNF alpha agents such as adalimumab.

On the contrary, Lichtenstein *et al*^[146] pooled data from multicentre prospective randomised controlled trials in adult patients with IBD and concluded that concomitant use of immunomodulators-principally thiopurines but also methotrexate in isolated cases with CD did not significantly increase efficacy or alter thiopurine safety; infusion reactions were about 50% less in combined therapy than in infliximab monotherapy.

Van Assche *et al*^[147] conducted an open label randomised controlled trial to evaluate the influence of anti-TNF alpha discontinuation after patients had been in remission for at least six months with combined therapy; no clear benefit of continuing combined therapy beyond six months from clinical remission was demonstrated.

Combination of anti-TNF agents with thiopurines may therefore enhance immunosuppression, however the risk of related adverse drug reactions and toxicity is also enhanced^[148]. Combination therapy may also be associated with higher relative risk of opportunistic infections in UC, but no significantly increased absolute risk of serious infections has been observed^[58,149].

Limited evidence exists on drug interactions between azathioprine and adalimumab. The latter has recently obtained approval by the European Medicines Agency and the United States Food and Drug Administration for use in adult patients with moderate-to-severe, active, refractory UC, who are intolerant to corticosteroids and thiopurines^[150]. Adalimumab is effective in inducing and maintaining remission in patients with active, moderate-to-severe, luminal or perianal CD, or patients with previous loss of response or intolerance to infliximab^[151-153]. A recent prospective case series of twelve adult patients with CD reported that 6-TGN and 6-MMPR were not influenced by concomitant administration of adalimumab during a 12 wk follow up period^[154]. The same study reported no change in TPMT, ITPase or HGPRT enzyme activity after 4 wk of combined treatment. More research is required into the efficacy and safety of combination treatment.

THIOPURINE TOXICITY: IS ALTERNATIVE THIOPURINE USE SAFE?

Chaparro *et al* has recently reported the findings of a large prospective nationwide cohort study from Spain, where 67% of 1026 patients had to discontinue thiopurine treatment due to adverse drug events such as nausea, arthralgia, alopecia, abdominal pain, liver and pancreatic toxicity, infection, leucopenia, myelotoxicity. 37% percent of them were restarted on the same thiopurine. 40% had recurrent side effects; 4% following treatment with the same thiopurine and 36% after introduction of an alternative thiopurine^[155]. Interestingly even in patients with severe complications such as hepatotoxicity, thiopurine re-introduction was tolerated in 74% of cases; abdominal pain recurred in 18% of cases, nausea and arthralgia recurrence close to 50% was noted. 85% of patients demonstrated recurrence of pancreatic toxicity, recurrence rates of infection and bone marrow failure were 80% and 65% respectively. Overall over half of the patients tolerated re-introduction of thiopurine treatment following drug induced side effects.

Ledder *et al*^[46] published a small case series of four adult patients with successful introduction of mercaptopurine following azathioprine induced pancreatitis.

A recent meta-analysis on alternative use of mercaptopurine in adult patients with IBD who suffered azathioprine-induced toxicity ($n = 455$ patients), concluded that favourable outcomes had been observed in two-thirds of patients where trial of mercaptopurine was implemented^[57]. In detail, 62% with gastrointestinal toxicity, 81% with hepatotoxicity and 36% with flu-like illness had been able to tolerate mercaptopurine. Trial of mercaptopurine is therefore advised in cases of azathioprine intolerance, except in patients with severe pancreatitis or bone marrow aplasia. Among patients who discontinued mercaptopurine for further adverse effects, 59% experienced the same adverse effect as they had with azathioprine.

Further studies are required so as to quantify the hepatotoxicity risk associated with thioguanine as an alternative therapy for IBD treatment; metabolism of this non-conventional thiopurine does not generate 6-methyl mercaptopurine^[156].

VALUE OF PHARMACOGENETICS IN THIOPURINE SAFETY MONITORING

Observed genetic polymorphisms have been reported to play pivotal role in the occurrence of adverse drug reactions for various drugs, including thiopurine^[157,158], warfarin^[159], antiepileptic^[160] and anti-retroviral drugs^[161]. Inter-individual variability in drug response reflects differences in genetic polymorphisms which, to some extent, may be responsible for variation in drug metabolism^[116,162].

TPMT is an extensively researched example of the clinical applicability of pharmacogenetics; pre-treatment testing is currently implemented; common variant alleles such as TPMT*2, TPMT*3A, TPMT*3C, TPMT*8 may

give rise to decreased enzyme production; heterozygous or homozygous TPMT deficient patients require decreased dose upon treatment commencement, or thiopurine avoidance respectively. Weinshilboum and Sladek first reported that approximately 0.3% Caucasians have complete deficiency, approximately 10% have low or intermediate activity and about 90% have high activity^[163]. Numerous studies have since verified the differences in TPMT levels and activity between different ethnic group^[164-166].

Pre-treatment determination of TPMT genotype and phenotype may be useful for prediction of thiopurine toxicity; TPMT testing is however not universally implemented by gastroenterologist^[167-169], because evidence on its predictive value for thiopurine toxicity in IBD is still unclear; Colombel *et al*^[170] has reported that the majority of adult patients with myelotoxicity had normal TPMT genotype. The controversy in the medical literature may exist because of the non-absolute concordance between genotype and phenotype (TPMT activity status)^[167,171], and the diversity in the laboratory methods (enzymatic, radiochemical, high performance liquid chromatography assays) used for quantification of TPMT activity in red blood cells^[172]. A systematic review by Booth *et al*^[173] reported that in patients with intermediate and low enzymatic activity, genotyping sensitivity to identify patients with low enzymatic activity ranged from 70.33% to 86.15% (lower-bound 95%CI: 54.52%-70.88%; upper-bound 95%CI: 78.50%-96.33%) and was therefore imprecise. Despite this finding, variant genotype and low TPMT activity were reported to be strongly associated with haematological toxicity.

Additional polymorphic genes implicated in thiopurine metabolism are under investigation for possible effect on effectiveness and safety. These are the ITPase^[174], the guanine monophosphate synthetase (GMPS mediates tIMP conversion to tGMP) and the glutathione S transferases (GST which catalyses MP production from azathioprine)^[175,176].

Smith *et al*^[177] reported that single nucleotide polymorphism (SNP) of aldehyde oxidase (AOX1) c.3404A > G may predict lack of response ($P = 0.035$, OR = 2.54, 95%CI: 1.06-6.13); when combined with TPMT activity, this information allowed stratification of a patient's chance of response to azathioprine, ranging from 86% in patients where both markers were favourable to 33% where both were unfavourable ($P < 0.0001$)^[177].

A common SNP, associated with a dramatically reduced ABCC4 function, has been identified in approximately 14%-18% of the Japanese population. In these patients, the OR of carrying the ABCC4 variant and having leucopenia following thiopurine introduction has been reported to be 3.30 (95%CI: 1.03-10.57; $P = 0.036$)^[178].

In the future, the pharmacogenetic approach may enhance pre-treatment safety and prompt dose adjustment^[179].

CONCLUSION

Safety of thiopurine treatment in IBD stipulates fine tun-

ing between therapeutic efficacy, intolerance and toxicity. This balance must be achieved on a long term basis. Combination therapy with new disease modifying drugs has further modified the safety profile of thiopurines; the importance of such interactions has yet to be confirmed in large studies across all age groups. There is a need for a standardised approach in therapeutic drug monitoring. Further research into disease pathogenesis and pharmacokinetic/pharmacodynamic pathways may identify potentially useful biomarkers for thiopurine safety monitoring. High quality, prospective and collaborative clinical research will establish a robust evidence foundation which will safely inform future clinical practice.

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Use of methotrexate in inflammatory bowel disease in 2014: A User's Guide

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Crohn's disease; Ulcerative colitis; Immunomodulators; Methotrexate user's guide

Core tip: Methotrexate can be a useful adjunct to the treatment of inflammatory bowel disease, but many practitioners are unfamiliar with its use. Here, we have provided a succinct summary of the data behind the use of methotrexate and a short "user's guide" and algorithm to allow for the busy clinician to become quickly familiar with the drug and information to help prescribe it safely.

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Abstract

Methotrexate has been used as an immunomodulator in many autoimmune diseases, including inflammatory bowel disease. However, many physicians are unfamiliar or uncomfortable with its use in the management of inflammatory bowel disease. We summarize the data for use of methotrexate in common clinical scenarios: (1) steroid dependant Crohn's disease (CD); (2) maintenance of remission in steroid free CD; (3) azathioprine failures in CD; (4) in combination therapy with Anti-TNF agents in CD; (5) decreasing antibody formation to Anti-TNF therapy in CD; (6) management of fistulizing disease in CD; and (7) as well as induction and maintenance of remission in ulcerative colitis. An easy to use algorithm is provided for the busy clinician to access and safely prescribe methotrexate for their inflammatory bowel disease patients.

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Key words: Methotrexate; Inflammatory bowel disease;

INTRODUCTION

Methotrexate (MTX) has a long history for effectively treating rheumatological conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and sarcoidosis^[1-3]. Over the past 25 years there have been numerous studies that evaluated its efficacy in Inflammatory Bowel Disease with varied results. It has to date remained in treatment algorithms as a salvage therapy for patients who have failed, or become intolerant of, azathioprine. The goal of our paper is to summarize the data behind methotrexate for common clinical situations and to provide a quick access guide on prescribing the drug.

MTX PHARMACOKINETICS

The landmark studies demonstrating efficacy of MTX in Crohn's disease (CD) have utilized *sq* or *im* at 25 mg/wk. Smaller non-randomized studies in both CD and UC patients have offered conflicting data and, to an extent

Table 1 Summary of methotrexate trials in Crohn's disease

Study	Dose MTX	Route of admin	n	Study design	Patients	Duration follow up (wk)	MTX response	MTX remission	Placebo or (Comparator) Response	AE MTX	AE Placebo
Kozarek	25 mg/wk	<i>sq</i>	14	Non-Randomized-open Label	CD	12	79%				
Feagan	25 mg/wk	<i>im</i>	141	Double-blind Placebo controlled multi center	Steroid dependent CD	16		39.4% ¹	19.1%	1%	2%
Oren	12.5 mg/wk	<i>po</i>	84	Randomized Double-Blind Placebo Controlled	Active CD	36		38%	46%		
Arora	22.5 mg/wk	<i>po</i>	33	Randomized Double Blind Placebo Controlled	Steroid Dependent CD	52	54%		20%	23%	0
Feagan	15 mg/wk	<i>im</i>	76	Double Blind Placebo Controlled Multi-Center	CD Maintenance	40		65% ¹	39%	1%	2%
Mate-Jimenez	15 mg/wk	<i>po</i>	38	Randomized Single Center	Steroid Dependent CD	76		80% ¹ Induction 66.6% ¹ Maintenance	14% Induction 0 Maintenance	11.5%	0
Lemann	25 mg/wk	<i>im</i>	49	Retrospective	Active CD			84%		49%	
Fraser	20 mg/wk (10-25)	<i>po/im</i>	48	Retrospective	Active CD-Maintenance			62%		27%	
Ardizzone	25 mg/wk	<i>iv</i>	54	Investigator Blind, randomized	Active CD	24		56%	63% AZA	11%	
Mahadevan	25 mg/wk	<i>im</i>	16	Retrospective case series	Fistulizing CD		56%			6%	
Wahed	25 mg/wk Induction 15 mg/wk Maintenance	<i>im/po</i> -Induction <i>po</i> -Maintenance	99	Retrospective	AZA Intolerance/ AZA non-responders		62%			8.3%	
Feagan	Wk0-10 mg/wk Wk3-20 mg/wk Wk5-25 mg/wk	<i>sq</i>	126	Double Blind Placebo Controlled Multi-center	Active CD	50		IFX + MTX 56%	IFX + PCBO 57%		

¹*P* < 0.05 *vs* MTX response. MTX: Methotrexate; CD: Crohn's disease; AE: Adverse events; AZA: Azathioprine.

demonstrate, the relative ineffectiveness with low dose *po* regimens for induction or maintenance of remission (Table 1)^[4,5]. Jundt demonstrated similar bioavailability between *po vs sq vs im* MTX in RA patients^[6]. The bioavailability of *po* as compared to *im* was 0.85.

Kurnik *et al*^[7] studied the bioavailability of MTX in adult patients with stable Crohn's disease. The patients were administered their weekly doses either orally or *sq* and the MTX levels were measured over the next 24 h. No information on extent of small bowel inflammation was provided. They found that oral bioavailability averages 73% (95%CI: 62%-86%) of that of subcutaneous administration^[7]. Hoekstra demonstrated that the bioavailability of *po* MTX can be boosted by split dosing. RA patients were studied after single dosing of MTX by either *sq* or *po* method. Then the same patient underwent a second measurement after split dosing of MTX (50% of the dose taken 8 h later). The bioavailability of the split

dose was 28% higher compared to the single dose (*P* = 0.007) and was statistically significant. The mean bioavailability after single-dose and split-dose MTX was 0.76 and 0.90, respectively, compared to subcutaneous administration^[8].

Wilson *et al*^[9] updated the Kurnik study using a more sensitive assay. They compared the pharmacokinetic profile of *po* and subcutaneous MTX (25 mg) in 11 CD patients. The bioavailability of *po* MTX compared with *sq* was found to be 0.86 (90%CI: 0.79-0.92). Of note, the 90%CI to meet definition of bioequivalency proposed by the FDA was not met, (lower end of the 90%CI would have had to be 0.80 rather than 0.79), and so this study could not claim true bioequivalency of the oral and *sq* routes of administration.

Although these are small studies and many patient factors were not provided (*i.e.*, extent and severity of bowel disease), the *po* route of administration does ap-

pear to be less bioavailable than *sq* dosing.

WHAT IS THE DATA FOR MTX IN INDUCTION OF REMISSION IN STEROID DEPENDENT CROHN'S DISEASE?

Although Kozarek *et al*^[10] (NEJM 1980) had demonstrated the efficacy of 6-mercaptopurine in the induction of remission of Crohn's disease, the authors noted the response to be delayed and incomplete. The first report of successful induction with methotrexate was reported by Kozarek *et al*^[10] in 1989. This non-randomized, open-label pilot study included 14 patients with Crohn's disease with an unidentified fraction described as failing immunomodulators. Eleven patients (79%) demonstrated a clinical response to 25 mg/wk *im* methotrexate as measured by objective decreases in CDAI, and 5 patients (36%) demonstrated endoscopic mucosal healing. Although this study lacked a control arm, it suggested MTX may have value in inducing remission in patients with Crohn's disease.

Feagan completed a prospective double-blind, placebo-controlled Canadian multicenter study of weekly *im* injections of methotrexate in patients who had chronically active Crohn's disease despite a minimum of 3 mo of prednisone therapy with the primary outcome being the induction of clinical remission^[11]. A total of 141 patients assigned in a 2:1 ratio of MTX to placebo were included in the trial and 37 (39.4%) achieved clinical remission in the methotrexate group compared with 9 (19.1%) in the placebo group ($P = 0.025$). The response among patients requiring high dose prednisone (> 20 mg/d) was equally good as those requiring low doses at study initiation. Prednisone dose was appreciably lower by week 4 in the MTX group and demonstrated the largest difference from week 12 through 16. A greater number of patients withdrew from the treatment arm due to adverse events (17% *vs* 2%). The withdrawals from the MTX arm were due to asymptomatic elevation of serum aminotransferase concentrations (7), nausea (6), skin rash (1), atypical pneumonia (1), and optic neuritis (1).

Oren *et al*^[5] conducted a prospective randomized, double blind, placebo-controlled Israeli multi-center trial to evaluate the effectiveness of oral methotrexate in patients who had required steroids or immunomodulators for at least 4 mo out of the year prior to enrollment. Although it would be difficult to characterize these patients as steroid dependant, they had active ongoing disease as measured by Harvey Bradshaw Index. The study randomized 84 patients to 12.5 mg *po* MTX/week *vs* 6-MP 50 mg/daily *vs* placebo. The lower dose of oral MTX (compared to 25 mg/wk *im* in the Feagan study) was based on reported efficacy in the rheumatoid arthritis literature. Remission rates were 39% and 41% in the MTX and 6-MP groups respectively. However, the rate of remission in the placebo group was 46%, thereby inferring no benefit for either the MTX or 6 MP treatment arm.

Criticisms of this study included presumed underdosing of MTX and 6 MP. Also, no standard steroid tapering regimen was described in this study, although reduction in steroid dose was described as an outcome measure. Although improvement was seen based on intra-patient evaluation (each patient used as their own control), this was not a pre-specified analysis. Hence, these results should be viewed with caution.

A cohort of 38 patients with steroid dependant CD was evaluated by Mate-Jimenez, but the requirement to separate these patients into 3 arms (1.5 mg/kg per day 6MP, 15 mg/wk *po* MTX, or 5-ASA) resulted in a small number of patients in each arm^[12]. However, the large differences in outcomes for induction of remission in both treatment arms (93.7% 6MP, 80%MTX) compared to placebo (14%) was statistically significant. Interestingly, these findings show a degree of benefit that has not been reproduced for either the 6MP or MTX treatment arms. Arora *et al*^[13] evaluated 28 steroid-dependant Crohn's disease patients who received 15 mg/wk *po* MTX *vs* placebo. Dose escalation to 22.5 mg/wk was allowed at the discretion of the clinician. The primary endpoint was clinical exacerbation of Crohn's disease. Although fewer patients in the MTX group (6/13, 46%) experienced exacerbation of CD *vs* placebo (12/15, 80%), the findings did not reach statistical significance. Despite the 43% relative risk reduction in flare frequency between the treatment and placebo, this study was underpowered to find this difference to be significant.

Ardizzone evaluated the efficacy of *iv* MTX in comparison to AZA^[14]. This randomized investigator-blind study enrolled 54 steroid-dependent active (CDAI > 200) CD patients on > 10 mg/d of steroid therapy. Patients were randomized to 25 mg *iv*/wk of MTX *vs* *po* AZA 2 mg/kg per day for 3 mo, after which MTX dosing was changed to 25 mg/wk *po* for an additional 3 mo follow up. The primary outcome considered was the proportion of patients entering steroid-free remission after 3 and 6 mo of therapy. No statistically significant difference was found between the two treatment regimens with respect to remission rate after 3 mo (methotrexate 44%, azathioprine 33%, $P = 0.28$, 95%CI: 0.369-0.147), and 6 mo (methotrexate 56%, azathioprine 63%, $P = 0.39$, 95%CI: 0.187-0.335), respectively. MTX and AZA demonstrated similar rates of adverse events leading to medication withdrawal. While there appeared to be no additional benefit to providing MTX *via* the IV route, MTX at 25 mg/wk appeared to have similar efficacy as weight based azathioprine in inducing and maintaining remission in active Crohn's disease.

A 2011 meta-analysis of MTX in active Crohn's did not include either the Mate-Jimenez or Ardizzone studies (no placebo arm) or Arora studies (categorized the study patients as quiescent)^[14]. Their conclusion that MTX was not better than placebo in active Crohn's was based only on the inclusion of Feagan's positive trial (25 mg/wk *im* MTX) and the negative orally administered MTX (12.5 mg/wk *po*) Oren trial. The Cochrane collaboration

reached similar conclusions a year later, but understood the limitations of the data on oral MTX and suggested further study^[15].

WHAT IS THE DATA FOR MTX IN MAINTENANCE OF STEROID-FREE REMISSION IN CROHN'S DISEASE?

Feagan demonstrated the use of MTX in Crohn's disease for maintenance of remission in a large double-blind, placebo controlled multi-center study with 76 patients in 2000^[16]. Some of these patients were enrolled from Feagan's trial for induction of remission using 25 mg *im*/wk MTX in 1995 and others from an open label trial of 25 mg/wk *im* MTX. The patients were randomized to 15 mg *im* MTX/weekly *vs* placebo and followed for 40 wk. Impressively, no other therapy for Crohn's disease was permitted. At the completion of the trial 65% (26/40) of the MTX group maintained remission compared to 39% (14/36) of the placebo group ($P = 0.04$). A majority (55%) of the relapsers could be re-induced with 25 mg/wk *im* MTX. Adverse events were minimal as only 1 patient discontinued MTX therapy for nausea and vomiting.

The efficacy of oral MTX (10-20 mg *po*) for maintenance of remission in Crohn's and ulcerative colitis was evaluated by a retrospective review by Fraser. Although 1 year remission rates approached 90%, the data for Crohn's and UC were combined and the clinical definition of remission was vague^[17].

Given the dearth of high quality studies of MTX in maintaining remission in Crohn's, the only maintenance study used in the Kahn meta-analysis was Feagan's (15 mg *im*/wk MTX) suggesting benefit with a number needed to treat (NNT) of 4^[14]. Interestingly, the Cochrane meta-analysis of MTX for maintenance of remission, included both the Mata-Jimenez study and Oren studies as part their analysis^[18]. Their main conclusions track the benefit shown by the Feagan's 15 mg/wk *im* MTX and suggest that lower oral doses do not benefit maintenance of remission.

CAN MTX BE USED IN PATIENTS WHO FAIL AZA AND HOW DURABLE IS THE RESPONSE TO MTX?

Despite the widespread use of thiopurines, approximately one third do not respond and another 10% cannot tolerate the drugs^[19]. In the United States, MTX is often reserved for AZA intolerance or failure and fewer physicians are comfortable prescribing it^[20]. AZA Intolerance can include bone marrow suppression, upper GI symptoms, pancreatic dysfunction, abnormal LFT's and nonspecific symptoms including joint aches, hair loss, rash and flu like illness.

A study by Lemann in 2000 evaluated the durability of MTX for maintenance of remission in a population of patients who had (mostly) failed or were intolerant to

AZA and had *already been treated* with MTX for period of at least 6 mo were followed for an additional 18 mo^[21]. Out of 49 patients, 42 had previously failed AZA (85%). Out of the 41 achieving remission, 36 had previously failed AZA (87%). Most of the patients were administered 25 mg/wk *im* MTX, but some physicians changed the dose to oral administration and some were even able to taper it. Despite some patients with oral MTX dosing and despite a heavy proportion of AZA failures in the study population, 71% of the study population remained in remission for 1 year and up to 52% remained in remission after 3 years. Among patients who initially do well on MTX after AZA failure, they are likely to remain well on that therapy over the next several years.

Wahed *et al*^[22] evaluated clinical response of 99 CD patients retrospectively who were placed on MTX due to AZA intolerance or nonresponse. The study suffers from a non-homogenous doses and method of administration of MTX for induction and maintenance. The range of induction dose of MTX was 2.5-25 mg/wk and administration varied as either *im* or *po*. Improvement was based on multiple variables as available from the charts, but was not standardized. With these caveats, clinical response occurred in 18 of 29 patients (62%) refractory to AZA/MP and 42 of 70 patients (60%) intolerant to AZA/MP. This suggests that MTX is effective in CD patients previously treated with AZA who experienced failure or non-response.

At present, there are no high quality trials (prospective, identical induction doses and method of administration, presence of control groups) on which to confidently choose to use MTX specifically in a population of AZA/6MP failures, but it would not be unreasonable to attempt MTX.

DOES COMBINATION MTX AND ANTI-TNF THERAPY TO TREAT CROHN'S DISEASE RESULT IN BETTER OUTCOMES?

The landmark SONIC study demonstrated that patients with moderate-to-severe Crohn's disease who were treated with combination infliximab plus azathioprine were more likely to have a corticosteroid-free clinical remission than those receiving azathioprine or infliximab monotherapy^[23]. Concomitant immunosuppressive therapy also reduces the magnitude of the immunogenic response of infliximab^[24]. It follows that methotrexate, as part of combination therapy with anti-TNF agents, may provide similar benefits.

Feagan *et al*^[25] studied this hypothesis in the COMMIT trial. They performed a 50-wk double-blind, placebo-controlled trial of MTX + IFX *vs* IFX monotherapy in Crohn's patients who had started prednisone therapy within the preceding 6 wk. Patients were not permitted to use any other therapy with the exception of antibiotics for 14 d in the case of active perianal disease. Patients

were initiated on IFX 5 mg/wk and 10 mg *sq* MTX/week (escalating to 25 mg/wk by week 5) or IFX 5 mg/wk and placebo injections. Prednisone was force tapered in all patients by week 14. The primary outcome evaluated steroid free-remission by week 14 or maintenance of remission by week 50. Steroid-free remission at week 14 was 76% (48/63) in combination therapy compared to 78% (49/63) with IFX mono therapy ($P = 0.83$). At week 50, 56% (35/63) *vs* 57% (36/63) maintained remission in the combination arm *vs* monotherapy arm. Mean methotrexate doses at week 50 in the treatment arm was 22.3 mg/wk. This study found that combination therapy with IFX and MTX had no more benefit than IFX alone.

Based on the strongest current body of evidence (SONIC, COMMIT), it seems reasonable to prefer combination therapy using AZA/6MP rather than MTX in those Crohn's patients able to tolerate it.

IS MTX EFFECTIVE IN PREVENTING AUTO-ANTIBODY FORMATION WHEN USED IN COMBINATION WITH BIOLOGIC THERAPY?

A prospective study by Vermeire evaluated the development of antibodies to infliximab (ATI) when combined with AZA, MTX, or placebo^[26]. The concomitant use of immunosuppressive therapy (MTX or AZA) was associated with a lower incidence of antibodies to IFX (53/115, 46%) compared with patients not receiving concomitant immunosuppressive therapy (43/59, 73%; $P < 0.0001$). Furthermore, the incidence of antibody formation was not different between the MTX and AZA groups, 44% compared to 48% respectively. Patients not taking IS therapy had lower IFX levels (median 2.42 mcg/mL) 4 wk after any follow-up infusion than patients taking concomitant IS therapy (median 6.45 mcg/mL) ($P = 0.065$), but there was no difference between MTX or AZA. Sokol *et al*^[27] confirm that patients using co-treatment with immunosuppressives experienced less IBD activity and less need to switch Anti-TNF therapy due to secondary loss of response. In fact, their data suggest efficacy of AZA over MTX, though their patient population included both CD and UC patients, and it is not clear whether any of the UC patients were treated with MTX and included in the analysis.

Although the COMMIT study did not show an improvement in 50 wk outcomes using combination therapy (IFX + MTX *vs* IFX alone), the MTX combination group did achieve statistically significant lower antibody levels (4% compared with 20%, $P = 0.01$) and demonstrated higher median serum trough levels of IFX (6.35 µg/mL *vs* 3.75 µg/mL, $P = 0.08$), similar to what is seen with azathioprine combination therapy^[25]. Whether this would result in fewer instances of infusion reactions or secondary non-response to IFX beyond 50 wk remains to be seen.

CAN MTX BE USED TO MANAGE SECONDARY NONRESPONSE TO BIOLOGIC MONOTHERAPY?

Absah retrospectively evaluated 14 pediatric patients with moderate to severe (CD) eventually failing anti-TNF- α therapy (13 ADA and 1 IFX) who then received concomitant methotrexate (median dose 17.5 mg *sq*/wk)^[28]. Most (12/14) patients had also previously failed AZA therapy (though it is not made clear whether this was as part of combination with biologic). Clinical remission was achieved in 7/14 (50%) of patients on average of 6 wk after MTX initiation with no additional improvement in the other 7 patients during 10 mo of follow up. Unfortunately, no levels of biologic or antibody to biologic were measured in this study, so the mechanism of improvement remains unknown. Further research focusing on the adult population along with mechanism of action would serve to direct therapy in this refractory population often seen in tertiary centers.

DOES MTX TREAT FISTULIZING CROHN'S DISEASE?

To date, only small retrospective series are available to evaluate the efficacy of MTX monotherapy in fistulizing Crohn's disease. A research conducted a retrospective chart review of all Crohn's disease receiving methotrexate 15-25 mg *im* MTX/weekly. This group of patients that had failed or were intolerant to 6MP and were made up of perianal fistulae (9), abdominal wall (3), rectovaginal (1), bladder (1), perianal + rectovaginal (2). Overall, 4/16 (25%) experienced complete fistula closure and 5/16 (31%) had partial fistula closure. Fourteen of sixteen patients received full dose 25 mg *im*/wk of MTX for 3 mo and were switched to *po* for maintenance. The time to response could not be determined in half of the patients, but ranged from 4-13 wk in the other half. Another study found that 8/18 (44%) patients with Crohn's-related fistulas achieved partial or complete response using MTX for 6 mo, but information about success and failure based on oral or *im* administration was not provided^[29]. A pilot study of 12 patients using combination infliximab and MTX found 7 patients had total or partial response to fistula, but there was no MTX only arm and the data seem similar to the benefit achieved with IFX monotherapy^[30,31].

Approximately 10% of peri-anal and abdominal fistulas in Crohn's heal spontaneously^[31]. Given a closure rate well above the spontaneous closure rate, we consider MTX a potentially useful adjunct in management of Crohn's fistulas.

METHOTREXATE AND ULCERATIVE COLITIS

Does MTX work for induction of remission in UC?

Evidence pertaining to the utility of methotrexate in

Table 2 Evidence for induction of remission of ulcerative colitis with methotrexate

Study	Dose (mean)	Route	No. of patients	Study design	Follow-up (wk)	MTX response	MTX remission	Placebo response
Kozarek	25 mg	<i>im</i>	7	Open label	12	5/7 (71.40%)		N/A
Baron	15 mg	Oral	8	Open label	18	3/8 (37.5%)	0	N/A
Oren	12.5 mg	Oral	67	Placebo control	36	14/30 (46.7%)		18/37 (48.6%)
Egan	15 mg	<i>sc</i>	18	Open label	16	7/18 (39%)	3/18 (17%)	N/A
	25 mg	<i>sc</i>	12			4/12 (33%)	2/12 (17%)	N/A
Mate-Jimenez	15 mg	Oral	34	6-MP control	30		7/12 (58.30%)	11/14 (78.6%)
Paoluzi	12.5 mg	<i>im</i>	10 thiopurine resistant/intolerant	Open label	26	10/10 (100%)	6/10 (60%)	N/A
Cummings	19.9 mg mean	Oral	11 AZA failure 31 AZA intolerant	Retrospective	30	3/11 (27%) 18/31 (58%)	14/31	N/A
Nathan	20-25 mg	<i>sc</i> / oral	23	Retrospective	N/A		11/23 (48%)	N/A
Wahed	10-25 mg	Oral, <i>sc</i>	9 thiopurine ineffective 23 thiopurine intolerant	Retrospective	26	7/9 (78%) 15/23 (65%)	N/A	N/A
Manosa	25 mg	Oral <i>sc</i>	7 33	Retrospective	26		24/40 (60%) remission	N/A
Saibeni	20 mg	Oral/ <i>sc/im</i>	23	Retrospective	N/A	11/23 (47.8%)		N/A
Khan	14 mg	Oral	68	Retrospective	60	25/68 (37%)		N/A
	25 mg	<i>sc/im</i>	23			7/23 (30%)		

MTX: Methotrexate; CD: Crohn's disease; AE: Adverse events; SC: Subcutaneous; PO: Oral; AZA: Azathioprine.

induction of remission for ulcerative colitis is conflicting (Table 2). Disparate results reflect disagreement over appropriate dosing and route of administration. To date, only one prospective, randomized placebo-controlled trial examining the efficacy of methotrexate in the treatment of ulcerative colitis exists; Oren *et al*^[5] in 1996 compared 12.5 mg oral methotrexate to placebo in the induction of remission of 67 patients with moderate/severe UC^[5,14]. All patients had active disease with a Mayo score of >7, and were taking steroids for at least 4 mo in the preceding year. The results were disappointing, with clinical remission rates of 46.7% (14/30) in the methotrexate arm in comparison to 48.6% (18/37) for the placebo arm, a non-significant difference. Of those who entered clinical remission, 64.3% of patients in the methotrexate arm had a relapse requiring steroid induction compared to 44.4% of placebo patients, again, an insignificant difference.

Overall, a low remission rate relative to placebo, long time to remission, and a high relapse rate in Oren's study all suggest a lack of efficacy for methotrexate in either the induction or maintenance of remission in ulcerative colitis. Of course, important criticism may be directed at the relatively low dose of MTX used and the oral route of administration.

Otherwise, a number of small open-label and larger retrospective analyses have been conflicting, not least due to differing definitions of response, length of follow up (12 wk-2 years), dose of MTX (7.5-25 mg/wk), and route administered (*po* vs *im*). None of these studies were considered of sufficient quality to be included in the meta-analysis by Khan *et al*^[14].

The most comprehensive of these was published last year by Khan *et al*^[32], presenting retrospective data regard-

ing experience with methotrexate in the Veterans Affairs (VA) system. A total of 91 patients with ulcerative colitis who were steroid dependent or refractory were commenced on oral (mean 14 mg) or parenteral (mean 25 mg) methotrexate. In the oral MTX cohort, 37% (25/68) were able to successfully wean from steroid therapy, compared to 30% (7/23) of the parenteral cohort.

Overall, looking specifically at induction of remission in ulcerative colitis, response to methotrexate ranged from 27%-100%, and remission rates ranged from 0%-63%. Considering the retrospective nature of most studies, it is impossible to determine the true impact of dose or route of administration. In prospective, open label or randomized controlled trials, response rates similarly ranged from 33%-100%, with remission rates ranging 17%-60%. There are no clear signals regarding the impact of dose, route of administration, or indication for step-up in therapy on remission or response rates in UC.

Does MTX work for maintenance of remission in UC?

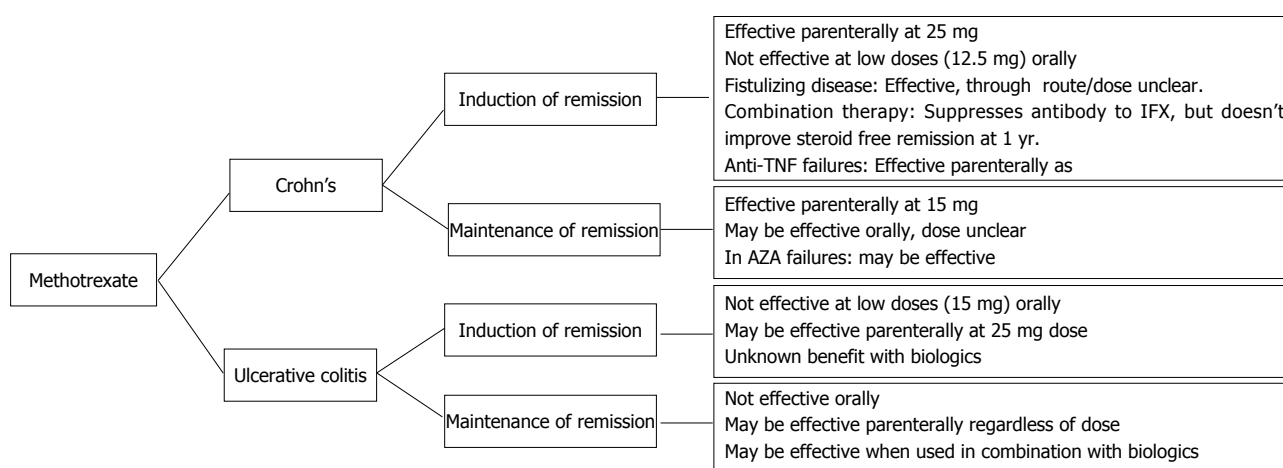
Regarding the maintenance of remission, the results are equally confusing - maintenance of remission rates range from 14%-75% (Table 3). Unfortunately, two open-labeled studies suggesting successful maintenance rates > 60%^[10,33] using parenteral methotrexate did not include a placebo arm as comparison^[10,33]. Oren *et al*^[5] and Mate-Jimenez *et al*^[12] included control arms, but provided disappointing results for the efficacy of oral methotrexate. Whether the route is a factor for better response rates remains to be seen.

There has been no data to date investigating the utility of combining methotrexate with biologic therapy in UC. Increasing interest in using methotrexate as a "synergistic enhancer" - to augment and prolong biologic efficacy -

Table 3 Evidence for maintenance of remission of ulcerative colitis with methotrexate

Study	Dose (mean)	Route	No. of pts	Study design	Follow-up period (mo)	MTX response maintained?	Control response	Significantly effective?
Kozarek	> 7.5 mg	sc	5	Open label	24	3/5 (60%)	N/A	N/A
Oren	12.5 mg	oral	32	Placebo-controlled	9	5/14 (36%)	10/18 (56%)	No
Mate-Jimenez	15 mg	oral	12	6-MP control	18	1/7 (14%)	7/11 (64%)	No
Paoluzi	12.5 mg	im	10	Open label	24	6/8 (75%)	N/A	N/A
Manosa	25 mg	Oral/sc	7/33	Retrospective	24	35%		N/A

MTX: Methotrexate.

**Figure 1** Algorithm for evidence-based use of methotrexate in inflammatory bowel disease. AZA: Azathioprine.

may help define its role in this disease.

PRACTICAL ADVICE ON HOW TO PRESCRIBE MTX IN THE US

Injectable MTX is available in 50 mg/2 mL vials. We prescribe one vial (2 loading dose equivalents) as well as a supply of “tuberculin” 1 mL syringes with 27 gauge, 1/2” needles. The patient draws 25 mg weekly from the vial and injects subcutaneously in either lower quadrant of the abdomen or inner thighs as their preference. After 12 wk, if they have a response, they can be transitioned to oral methotrexate maintenance. A patient friendly resource on injecting MTX is available *via* the Canadian rheumatology association (http://rheuminfo.com/wp-content/uploads/2011/04/METHOTREXATE_INJECTION_SHEET.pdf).

Oral methotrexate is available in 10 and 15 mg strengths as Trexall™. If using oral methotrexate in the induction of remission of IBD, we would recommend starting with 25 mg weekly, reverting to the subcutaneous route in non-responders and those who develop nausea attributed to the oral route.

All patients should be prescribed folic acid 1mg daily as it significantly reduces hepatic toxicity, an infrequent occurrence, and gastrointestinal toxicity associated with MTX^[34,35]. At present, our target population for MTX

are CD patients who are unable to tolerate azathioprine or 6Mercaptopurine due to adverse events, homozygous TMPT mutations, or inefficacy. In the event that methotrexate is required in a woman of child bearing age, we counsel regarding the need for effective contraception (*i.e.*, IUD) and recommend a discussion with their obstetric physician. We advocate obtaining routine blood labs (complete blood count, basic chemistry panel, hepatic function panel) 1 wk after initiation as well as every 8-12 wk subsequently.

CONCLUSION

Given the current evidence an algorithm for MTX can be elucidated (Figure 1). Providers should no longer shy away from using MTX due to concerns of hepatotoxicity and intolerance. Methotrexate demonstrates a similar rate of drug withdrawal as AZA, and may be considered favorable in young males in whom practitioners are reluctant to use AZA (due to concerns of hepato-splenic T-cell lymphoma risk). Determining the optimal dose and route of administration in the various indications for use in IBD is the current priority. MTX is largely used as a second line therapy after AZA failure. It may be useful in combination with Anti-TNF therapy to reduce the risk of immunogenicity and subsequent secondary loss of response to anti-TNF therapy. We eagerly await the results

of two studies that will shed further light; the METEOR trial and MERIT-UC, both randomized, controlled trials of parenteral MTX 25 mg weekly in the induction and maintenance of remission in steroid dependent or refractory ulcerative colitis.

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Inflammatory bowel diseases: Current problems and future tasks

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Abstract

Current knowledge on inflammatory bowel disease (IBD) is mainly endorsed by controlled trials and epidemiologic studies. Yet, we seldom look at the messages from real-world practice. Among a patient population followed since 2008, we looked at an unselected sample of 64 IBD patients [26 Crohn's disease (CD) and 38 ulcerative colitis (UC)] who had been seen as out-patients in the last year. Inducing remission, mesalamines (86% for UC/69% for CD/33%-16% as MMX formulation) prevailed as prescriptions; steroids (55%/19% for UC/CD) ranked second. Prescription of third-party drugs (antibiotics, NSAIDs, biologics) and adherence, were issues in the maintenance. 34% of CD, and 23% of UC patients showed accompanying immunologic diseases: CD-associated familial psoriasis (4:9) ranked first. **Main Message.** The association between IBD (CD mainly) and psoriasis, now found in our practice, matches current basic science gathering IBD together with psoriasis (and perhaps chronic respiratory disease) under the comprehensive term "barrier organ disease" wherein an epithelial surface with sensor systems rules contacts between outer antigens and a reactive underneath tissue, with the balance between inflammation and quiescence kept at any time by mucosal permeability. IBD is thus viewed as a polyfactorial/polygenic/syndromic

disorder, embedded into a galaxy of immune conditions offering multiple points of attack. This mindset of splitting the IBDs into pathogenic categories may allow overcoming the uniformly targeting of a single cytokine by biological drugs, in favor of demarcating the boundaries between different disease-subtype-specific indications, and paving the way to future personalized strategies.

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Key words: Inflammatory bowel disease; Immunopharmacology; Barrier organs; Future trends in inflammatory bowel disease; Microbiome

Core tip: Long after their description, ulcerative colitis and Crohn's disease (IBD) are still treated but not cured. This somber spell has now begun to be broken by genetic discoveries and by the study of the human microbiome. The former have uncovered hundreds of genetic variants lending support to the clinical hint that IBD is a syndrome encompassing discrete polymorphisms of the immune response pathways, each requiring a personalized approach. The latter has shown the microbiome to be a cell universe which, if disrupted, can provoke IBD together with a myriad of disturbances apparently unrelated with the gut. A frame of mind seeing the IBDS as embedded into a plethora of genetically linked immune disturbances must fuel IBD research from now on.

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STUDY SETTING AND SCOPE OF THE ANALYSIS

Supported by the Italian Health System, in 2008 Grad-

Table 1 Contains patients' demographics and disease characteristics

UC (38, 24 m)		CD(26, 16 m)	
Age, yr	Extension	Age, yr	Extension
18-80	Proctitis, 12	16-73	Ileo-colonic 15
	Sub-total, 11		Colitis, 5
	Left, 8		Universal, 4
	Pancolitis, 6		Ileitis, 2
	IPAA, 1		

UC: Ulcerative colitis; CD: Crohn's Disease; IPAA: Ileo-pouch anal anastomosis.

Table 2 Gives the frequencies of use of the main drugs *n* (%)

Ulcerative colitis	Crohn's disease
Mesalamines 33 (86)	18 (69)
Steroids 21 (55)	5 (19)
Thiopurines 14 (36)	8 (30)
Biologics 1(2.6)	1 (3.8)

enigo Hospital has launched an out-patient service mainly devoted to patients with inflammatory bowel disease (IBD). An interim analysis of the activities of this service has already appeared in 2010^[1]. Eversince its establishment, the service has mostly been conducted by one of us (GCA), enrolling some 200 IBD patients. The scope of the present analysis was to reappraise the data under the light of modern achievements (for example the concept of "barrier organ disease"); to gain more insight into the drawbacks and the limits of traditional therapy with special regard to factors countering maintenance of remission; then, to cast a glimpse into the future of treatment approaches for IBD. We deliberately meant to not loose adherence to our daily clinical experience in this out-patient setting, when either dissecting actual difficulties or visualizing future therapeutic scenarios (personalized treatment for example). At a time when the literature is being "flooded" by a number of large epidemiologic and population studies, we chose to present the limits and the peculiarities of a study that pivots on the narrow environment of an outpatient office conducted by one physician.

STUDY POPULATION

Sixty-four IBD patients, gathered in the most recent interim analysis between 6.6.2012 and 04-24-2013 included 26 Crohn's affections (CD) and 38 ulcerative colitis (UC) cases, corresponding to some 6 IBD patients per month; overall analysis in the previous 31 months had yielded 119 IBD patients. Changes in the core storage system beginning 2010 have imposed a discontinuity in the data collection modalities, a fault that is now mended (Tables 1-3).

Managing chronic remission: open questions

Both medical and budget issues make the maintenance of

Table 3 Illustrates the distribution of the main extra-intestinal affections

	<i>n</i>	Familial	Personal
Ulcerative colitis			
Psoriasis	2	0	2
Inflammatory bowel disease	3	3	0
Asthma	2	1	1
Rheumatoid Arthritis	2	0	2
Crohn's disease			
Psoriasis	4	3	1
Inflammatory bowel disease	3	3	0
Asthma	1	1	0
Rheumatoid Arthritis	1	1	0

remission of IBD a crucial challenge. The relevant literature has particularly expanded on UC^[2]. A variegated list of factors may provoke loss of IBD remission, and we ourselves had the chance to face some of the conditions in our real-world practice. (1) lack of adherence to prescriptions, mostly mesalamine and thiopurine medications. Among the 64 patients in this report, the adherence rate for mesalamines and thiopurines was found to attain 90% and 94%, ranking high with regard to literature data^[2]; (2) unavailability of a non-replaceable drug; we had to face this event for a few patients, who, owing to their intolerance of azathioprine, were prescribed 6-mercaptopurine, at a moment when the latter had become unavailable in our country (see below); (3) toxicity of a pivotal drug (mesalamine, azathioprine). Noteworthy, based on the results of an English survey which was able to reveal only 11 alleged cases of renal damage per million prescriptions, mesalamine is listed among the most tolerated drugs^[3]. Our own present series included a rare case of mesalamine-induced cholestasis^[4,5] which responded to patient's transitioning to balsalazide. As described in various publications^[6,7], we faced a rather common azathioprine toxicity. In a population of 42 UC patients and 37 subjects with CD (females mostly) we recently found an 11% of gastric intolerance to azathioprine. Transition to 6-MP was tolerated in 6 cases which acquired disease control^[8]; (4) undermining of remission because of the introduction of third party drugs: antibiotics and NSAIDs are mostly recognized as capable to reactivate IBD or induce it *de-novo*. Indeed, analysis of our office experience has gathered convincing evidence of a role for antibiotics and/or non-steroid anti-inflammatory drugs (NSAIDs) in active episodes of IBD, requiring the consideration of prescribing physicians^[9]. A specific attention must be devoted to the Crohn's-like colitis^[10] that is not rarely found as an accompaniment to immune-mediated diseases from rheumatoid arthritis to multiple sclerosis: its inciting factors have been recognized in anti-tumor necrosis factor (TNF) formulations and/or rituximab^[11], the impact matching the rising prescription rate of these drugs. In our opinion, these observation are an indicator of the pathophysiologic and genetic commonalities linking the IBDs with their surrounding galaxy of immune disorders of which psoriasis is just the most obvious instance; and (5) the issue of

the ancillary symptoms in IBD. Likewise any other individual, IBD patients may present with bowel abnormalities being due to a plethora of factors from irritable bowel syndrome to celiac disease. Such situations must be borne in mind, in order to avoid prescribing IBD drugs for the wrong indication (so-called over-treatment)^[12].

EXOGENOUS AND ENDOGENOUS FACTORING

Among variables factoring in the management of IBD, smoking is obviously the most studied, with a detrimental action being demonstrated for CD^[13], and a protective one for UC^[14]. Sometimes overlooked in clinical practice, passive smoking must by contrast be given adequate consideration. The causative role of NSAIDs and antibiotics has already been touched on.

Genomic instability is gaining crucial importance among endogenous factors in IBD management, with excessive frequency of hematologic or immune-allergic disorders in the patient or among his/her relatives.

PROGNOSIS

The anticipation that the IBDs that are followed in an out-patient environment might be benign is sometimes contradicted by data. Beginning 2008, for example, in our series we recorded at least three fatalities, including one hematologic malignancy, and two cases of septicemia. One drop-out patient was reported with colonic malignancy from another hospital.

WRAPPING UP SUMMARY

This data were gathered from a random sample of 64 IBD patients (38 UC, 26 CD), who were followed in the last year at an out-patient unit with a 5-year service history. Proctitis was common among the UC patients; mesalamines were the most prescribed drugs, with the MMX formulation attaining 16% in CD and 33% in UC; beclomethasone prescriptions were prominent among steroids, ranking to 12 prescriptions including 9 of local formulations; remission maintenance was a significant challenge, pivoting over two main aspects: the control of third-party drugs, and maintenance of adherence.

At least two patients on biologics presented with superimposed immune disorders: a young female receiving adalimumab for diffuse CD developed psoriasis of the sculp; a young male with juvenile rheumatoid arthritis received three different anti-TNF formulations and developed UC on each of the three^[15]; switched finally to certolizumab presented with psoriasis of the elbows.

The tables hint to an association between psoriasis and rheumatoid arthritis. Such clinical evidence in our opinion launches a few messages of a theoretical and clinical impact, and in the lines to follow we shall try to gain more insight into this matter.

Modern understanding of the anatomy of the gut and

of the pathophysiology of its associated immune system all convey a concept of the IBDs as disorders pivoting on a disrupted balance between the gut mucosal immune tissue and luminal antigens, with gut microbiota as one crucially causative variable in favoring or countering the rise of an inflammatory response; the underlying dogmatic view supporting this reasoning is that while the mucosal immune system has evolved following a tolerization tune, the submucosal lymphoid tissue is highly reactive and can mount a significant inflammatory response should any antigen breach the mucosal barrier.

IBD is now thought to best be described using a concept of a “contextualized syndrome”^[16]. The basis of this concept is double: (1) a uniform curative strategy for the IBDs is yet far from reach; and (2) though often presenting with obvious clinical commonalities, in fact the IBDs do hide distinct serological or genetic subtypes that are best accounted for by a process of splitting rather than one of lumping up^[17].

The frequent observation of a co-morbidity between IBD and psoriasis, such as that observed in our office, served as one of the triggers for this frame of mind. A part of the scientific community has thus begun to conceive IBD as an archetype of “barrier organ diseases” whereby the essential ingredients are a mucosal surface endowed with sensor molecules of the outer environment (see the NOD system for example), and an underneath lymphoid tissue, this mixing being ruled in the background by an abundant metagenomic microbiota load (see below).

At least three systems with similar characteristics have nowadays been defined in human beings: the gut (chiefly the colon); the skin; and respiratory epithelia. It is not by chance that clinical experience has long highlighted that disorders of these three districts might be co-morbid. Our case series recorded hereby emphasize a coincidence between CD and psoriasis, but others have written about chronic obstructive pulmonary disease and IBD^[18]. It is worth noting that the concept of barrier organ has been pioneered in 2005 by the brilliant work of Stefan Schreiber^[19]; the Italian research has recently contributed to this field by a comprehensive dermatologic review^[20] and by a gastroenterologic paper from our own^[21]. As to the state of the art, it seems uneasy to identify a morphological or molecular marker to distinguish those IBDs that associate with psoriasis from those which do not. A few years ago, a North-European group focused their attention on polymorphisms of the IL23 receptor (IL23R) in both IBD and psoriasis, thus perhaps envisaging a genetic link between the two disorders^[22].

Interest in the issue of the systemic positioning of IBD has been fostered by the increasingly frequent observation of ancillary immune diseases arising in patients on biologic treatments: development of IBD in rheumatic subjects receiving etanercept^[23], presentation with IBD of hematologic patients treated with rituximab^[24], and observation of psoriasis in cases of IBD prescribed adalimumab^[25]. The bulk of these observations implies the existence of a galaxy of immune-inflammatory con-

ditions (of which IBD is just one component) spanning from the gut to skin, lungs, and joints. The link between these conditions might be represented by anatomic/physiologic commonalities (barrier organ diseases) or a generic genetic instability perhaps sustained by polymorphisms of STAT transducers^[26].

This scenario recommends that the IBDs no longer be conceived as one nosographic entity. The bulk of the following observations: (1) NOD receptor polymorphism might drive CD phenotypes; (2) there is a link between serologic subtypes and clinical presentations; and (3) some CD presentations do depend on ethnic factors. All of these data contribute to build up a vision of IBD like a non-dichotomic collection of different (though linked) entities that are best described using the definition of “syndrome”^[16].

The implications of this changed frame of mind cannot be ignored. If it is understood that the entity “IBD” contains in fact multiple distinct syndromes along a clinical-serologic-genetic axis, then this must somehow be reflected in differentiated clinical interventions. Such a cutting-edge frame of mind can now hardly fit the widespread recommendation and use of biologic approaches^[27], which target one cytokine in an homogenized-pragmatic attempt to interfere with the common downstream pathways in the mechanisms of IBD.

A GLIMPSE INTO FUTURE TARGETS TO STUDY AND TREAT THE IBDs

Attempts to ensure “sealing” of the gut mucosa with the scope to limit contacts between the immunogenic luminal content and the lymphoid tissue underneath. Partial results of an approach using phosphatidylcholine have already been published^[28].

Triggered by the classic evidence that germ-free animals do not develop IBD, investigators could not neglect the colonic microbiota, which constitutes a heavier meta genome than somatic cells themselves. Various attempts to modify the amount and composition of colic metagenoma have thus proliferated: (1) oral administration of pro-biotic lysates^[29]; (2) fecal transplants^[30]; and (3) diet modifications^[31].

The data from the bulk of these studies is conveying the message that a quantitative or qualitative change of gut microbiota colonization (dysbiosis) might associate with a plethora of (auto)immune and (auto)inflammatory disorders^[32], with a particular emphasis on rheumatoid arthritis (RA)^[33]. Relevant cutting-edge results^[34] are now showing that *Prevotella Copri* (an in-habitant species of the microbiome) might train T-lymphocytes to secrete IL-17, a key mediator in the pathogenesis of RA. To this end, attention is concentrating on the recent claim that NOD receptors on colonic epithelial cells (whether tolerant or reactive against colonic flora at birth) might drive the metagenomic phenotype of the newborn: rather a breakthrough, in view of the ability of colonic species to condition a whole array of affections, from IBD itself to

hepatic steatosis^[35].

Research directed to identify and change factors in the genesis of IBD, such as life style and diet composition^[36].

Along a totally different line, the results have been published of attempts at unraveling genetic IBD surrogates, that though mimicking IBD, might atypically respond according to the signal conveyed by the hidden gene: Behcet mimicking IBD^[37] and familial mediterranean fever are instructive example^[38].

CONCLUSION

Though generated in a limited environment, the analysis of the data from our office has led to general considerations. The IBDs can no longer be considered as autonomous entities, but rather as poly-organic and poly-genic syndromes wherein a critical mass of polymorphic genetic information and environmental factors must interact for full-blown disease to develop^[39]. Visualizing the IBDs like archetypes disorders of the immunological interaction between the “in” and the “out” (together with skin and pulmonary epithelia disorders) to make the umbrella label of “barrier organ disease” seems particularly seminal. This novel positioning of IBD might at first sight increase the degree of complexity, but on the other hand can favor novel therapeutic approaches and pave the way towards the conception of a personalized therapy.

Though apparently stable in the Western World, IBD has two formidable avenues to run. Firstly, Far East populations seem no longer to be immune from the IBDs, and in the next few years may witness an epidemic explosion of these disease^[40]; secondly, populations that immigrate to countries with a higher hygiene standard seem to be particularly prone to develop IBD^[41]. For certain countries, such challenges are not an issue of tomorrow, but are already here today.

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Protein kinases are potential targets to treat inflammatory bowel disease

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Abstract

Protein kinases play a crucial role in the pathogenesis of inflammatory bowel disease (IBD), the two main forms of which are ulcerative colitis and Crohn's disease. In this article, we will review the mechanisms of involvement of protein kinases in the pathogenesis of and intervention against IBD, in terms of their effects on genetics, microbiota, mucous layer and tight junction, and the potential of protein kinases as therapeutic targets against IBD.

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Key words: Inflammatory bowel disease; Protein kinase; Barrier function; Microbiota; Genetics

Core tip: The roles of protein kinases in the pathogenesis and intervention of inflammatory bowel diseases (IBD) are emerging. In this article, we will review the specific roles of different protein kinases in the pathogenesis of IBD, classify these protein kinases into different categories based on their fundamental functions in IBD, and describe substantial new mechanistic insights into the pathogenesis of IBD, highlighting protein kinases as potential intervention targets against IBD.

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD), two main forms of inflammatory bowel disease (IBD), are relapsing, idiopathic intestinal inflammatory conditions, caused by inappropriate and continuing immunologic responses to aberrant intestinal microorganisms in genetically susceptible individuals under certain environmental conditions^[1].

UC and CD differ^[2] with each other dramatically in different respects. UC is confined to the superficial area of the intestinal wall, whereas CD is transmurally distributed throughout the entire digestive tract but in a discontinuous way. The lesion is patchy with "lead pipe sign" in UC, but many polyps with "string sign" are often observed in CD. UC displays a Th2-like immune response, while CD shows a Th1 dominant response. Antineutrophil cytoplasmic antibodies were found in 65% of UC cases and 5%-10% of CD cases, and antibodies to yeast *S. cerevisiae* were found in 60%-70% of CD cases and 10%-15% of UC cases^[3]. Meanwhile, UC and CD share many similarities, such as neutrophil infiltration and epithelial barrier dysfunction. Despite the fact that there is no cure for IBD thus far, enormous progress about the pathogenic mechanisms of this inflammatory disorder has been around the corner in different aspects, such as genetics, regulatory immunology and microbiome.

The signaling pathways mediated by protein kinases have drawn much attention for connecting external stimuli including hostile environmental stresses with internal biological responses, such as intestinal inflammation. Protein kinases can be defined as enzymes which add phosphate

Table 1 Protein kinases related to inflammatory bowel disease genetics

Kinase	IBD	Ref.
ERK1	CD	[8]
p38	CD and UC	[9]
TYK2	CD and UC	[10]
JAK2	CD and UC	[11]
GCKR	CD	[12]
CDKAL1	CD	[13]
LRRK2	CD	[15]

ERK1: Extracellular signal-regulated Kinase; TYK2: Tyrosine kinase 2; JAK2: Janus kinase 2; GCKR: Glucokinase regulator; CDKAL1: Cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like; LRRK2: Leucine-rich repeat kinase 2; IBD: Inflammatory bowel diseases; UC: Ulcerative colitis; CD: Crohn's disease.

(called phosphorylation) to the side chain of serine, threonine or tyrosine of substrate molecules. This modification alters the biological function of the substrate, such as changing enzyme activity, cellular distribution, and even causing diseases^[4,5]. In this review, we will shed light on the roles of protein kinases in the pathogenesis of intestinal inflammation and describe some new mechanistic insights into the intervention of IBD, which targets at protein kinases.

PROTEIN KINASES AND GENETIC FACTORS

Genome-wide association studies demonstrated that genetic factors are very crucial in the individual susceptibility to IBD, for example, relatives of UC patients including twins display almost ten times greater risk of UC than non-relatives^[6,7]. As shown in Table 1, major IBD susceptibility regions on chromosomes 16 and 6 contain some genes encoding protein kinases like extracellular signals-regulated kinase 1 (ERK1)^[8] and p38^[9]. Several single-nucleotide polymorphisms in tyrosine kinase 2^[10] and Janus kinase 2^[11] were identified in IBD patients. Glucokinase regulator has also been associated with the risk of CD^[12]. The cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like plays an important role in susceptibility to CD, psoriasis and type II diabetes^[13,14]; leucine-rich repeat kinase 2 is identified to be related to the pathogenesis of CD^[15].

PROTEIN KINASES AND MICROBIOTA

Up to 10^{14} individual bacteria in the human gastrointestinal (GI) tract^[16], together with the mucous layer where the microbiome lives in, constitute the first line of defense in host against hostile external environment, modulating GI tract development, maintaining immune homeostasis, and regulating host metabolism rate. The bacterial abnormality plays a dominant role in the onset and development of IBD.

Commensal bacteria and host innate immune system

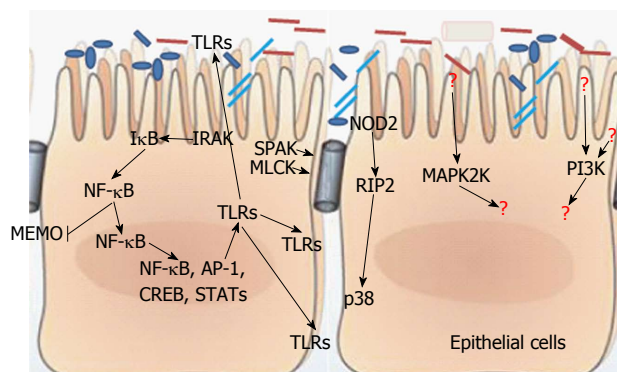


Figure 1 Intestinal epithelial cells use a variety of different molecules including protein kinases to monitor the presence of microbial pathogens, commensal bacteria, or host-generated products. Pathogen-recognition receptors, including TLRs, NOD2, and NLRs, are located on and within the cell where they recognize different threats. Recognition results in NF-κB activation, leading to the production of cytoprotective factors when stimulated by commensal bacteria and proinflammatory products when stimulated by potential pathogens, or blocks the activity of NEMO. Some other undefined factors can stimulate protein kinases such as PI3K or MAPK2K to regulate the process of intestinal inflammation. TLR: Toll like receptor; IRAK: Interleukin 1 receptor associated kinase; IκB: Inhibitor kappa B; NF-κB: Nuclear factor kappa B; SPAK: Ste20 like proline/alanine rich kinase; NEMO: NF-kappa-B essential modulator; MLCK: Myosin light chain kinase; CREB: cAMP response element binding protein; STAT: Signal transducer and activator of transcription; NOD2: Nucleotide-binding oligomerization domain-containing protein 2; NLRs: NOD-like receptors; RIP2: Receptor-interacting protein kinase 2; PI3K: Phosphoinositide 3 kinase; MAPK2K: Mitogen-activated protein kinases 2 kinase; AP-1: Activator protein 1.

evolve together and thus maintain mucosal immune homeostasis by balancing inflammatory responses and regulating a variety of bacteria-triggering signal transduction pathways^[17], such as uncoupling nuclear factor (NF)-κB or mitogen activated protein kinase (MAPK) dependent target genes in a negative feedback manner^[18,19]. The host's innate immune system is poised to be triggered by signs of bacterial challenge, specially, some pathogen-associated molecules such as flagellin, peptidoglycan, lipoteichoic acid, or lipopolysaccharide, together called pathogen-associated molecular patterns which can wake up the host innate immune system^[20,21] and be further sensed by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) or the nucleotide-binding oligomerization domain containing protein (NOD)-like receptors^[22] (Figure 1). These PRRs would then induce the activation of signaling cascades, mostly MAPK and NF-κB pathways. In terms of MAPK pathways, it follows MAP4K-MAP3K-MAP2K-MAPK pattern, and then, the activated MAPK undergoes translocation to the nucleus to activate molecules required for gene transcription, including inflammatory molecules^[23,24]. For example, anthrax toxin can induce macrophage death by inhibiting the p38 signaling pathway^[25,26], and MAPK-activated protein kinase 2 plays an important role in the pathogenesis of *Clostridium difficile*-associated intestinal inflammation^[27]. For the NF-κB pathway, after being activated by IκB kinase complex, it phosphorylates α subunit of IκB, the inhibitor of NF-κB. Phosphorylation of IκB, accompanied by its ubiquitination and proteolytic degrada-

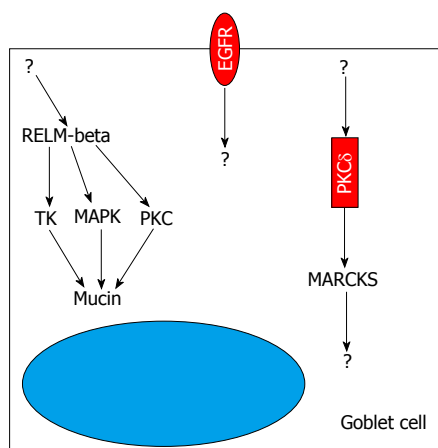


Figure 2 Intestinal Goblet cells employ different mechanisms including protein kinase related pathways to modulate the secretion of mucin, such as pathways related to tyrosine kinase, protein kinase C delta, myristoylated alanine-rich C-kinase substrate or receptors with tyrosine kinase activity such as epidermal growth factor receptor. MARCKS: Myristoylated alanine-rich C-kinase substrate; EGFR: Epidermal growth factor receptor; TK: tyrosine kinase; RELM-beta: Resistin-like molecule beta; PKCδ: Protein kinase C delta; MAPK: Mitogen activated protein kinase.

tion, results in exposure of the nuclear localization signal (NLS) on the now unbound NF- κ B^[28], which will further facilitate nuclear translocation of NF- κ B and be followed by transcriptional activation of many genes. In addition, even being regarded as an molecule which can promote inflammatory responses, an anti-inflammatory effect of NF- κ B was noticed; absence of NF- κ B essential modulator kinase causes spontaneous severe colitis, but commensal bacteria can stimulate the NF- κ B pathway to protect the host from exacerbating consequence^[29]. Blockage of epithelial NF- κ B pathway will deteriorate this colitis by increasing the translocation of bacterial to the mucosa^[30]. Besides the MAPK and NF- κ B pathways, some other signaling pathways are also very important, for example, after recognition of *Salmonella enterica* serovar *Typhimurium* curli fibrils in the gut, the TLR2-phosphatidylinositol 3 (PI3)-kinase pathway will be stimulated to tight the epithelial barrier^[31]. However, PI3 kinase signaling promotes *Campylobacter jejuni*-induced colitis through neutrophil recruitment in mice^[32]. RIP2 tyrosine kinase activity is required for NOD2-dependent autophagy process, but plays a dual role in this process. RIP2 sends a positive autophagy signal through activation of p38 MAPK and further relieves repression of autophagy mediated by the phosphatase PP2A^[33]. Not like NOD2 whose signaling induces cryptidins, MyD88-mediated TLR signaling induces RegIIIg and α -defensins, and more importantly, regulates bacterial infection-related mucosal immunity^[34-36]. In parallel, protein kinase C (PKC) can mediate the function of MyD88 adaptor-like (Mal) molecule in the maintenance of epithelial barrier integrity^[37].

PROTEIN KINASES AND BARRIER DYSFUNCTION

Basically, IBD is characterized by passive leaky diarrhea

and compromised intestinal barrier function. Except for the fact that commensal bacteria function as primary line of defense, protein kinases are also important in regulating the intestinal barrier function.

Mucus layer

The luminal side of the intestine is covered by a mucus layer which provides protection to the mucosa from mechanical damage and invasion of pathogens, and, together with commensal bacteria, constitutes a physical barrier between the epithelium and luminal contents including pathogenic bacteria, viruses, and parasites^[38,39]. This gel-like mucus layer can be divided by two distinguished layers-the outer and inner layers. The vast majority of intestinal bacteria, viruses and even parasites live in the flowing outer mucus layer; the inner layer is, however, an unstirred and relatively sterile layer adjacent to epithelial surface. The sterility of the inner layer accredits to the preservation of huge amounts of defensins, cathelicidins, and cryptidins with important function of anti-intestinal pathogens. Mucin coding gene *muc2*^{-/-} mice demonstrated spontaneous colitis because of increased transepithelial permeability^[40], in which bacteria can stick to the surface of the intestinal mucosa, which facilitates the translocation of bacteria into lower crypts and epithelial cells, thus triggering an inflammatory response^[39,41]. Protein kinases are involved in the integrity and maintenance of these mucus layers (Figure 2). Epidermal growth factor receptor (EGFR), harboring tyrosine kinase (TK) activity, has critical functions in development, growth, differentiation, proliferation and repair of epithelial cells^[42,43]. After stimulation by EGFR ligands such as transforming growth factor- α and epidermal growth factor, epithelial cells can develop into a mucous phenotype^[44,45]. However, inhibition of EGFR tyrosine kinase activity can abolish the effects of EGFR ligands on mucus production both *in vivo* and *in vitro*. PKC δ stimulates the secretion of mucin in the epithelium *via* regulation of myristoylated alanine-rich protein kinase C substrate pathway^[46]. Treatment of epithelial cells with PD98059 (MEK inhibitor) can inhibit MAPK activity and block the expression of terminal differentiation markers, such as sucrase-isomaltase, ITF, and MUC2, thereby interfering with the production of mucin^[47]. Some kinases like ERKs, TK, and PKC^[48] can regulate the production of mucin by mediating the activity of resistin and resistin-like molecule-beta; cathelicidin up-regulates MUC1 and MUC2 expression through MAPK pathway to modulate mucus synthesis^[49].

Protein kinase and epithelial junctions

The intestinal monolayer is characterized by polarization of apical and basolateral sides. The apical membrane is generally impermeable to hydrophilic solutes and contributes predominantly to mucosal barrier^[41]. Among the most important structures to determine paracellular permeability of the intestinal barrier are the epithelial tight junctions (TJs), which are made up of multiple proteins such as occludin and claudins^[50]. Occludin as the first

identified TJ^[51], plays an important role in epithelial/endothelial barrier integrity, and disruption of occludin regulation is an important aspect of a number of diseases^[52-54]. The claudins, as a group of TJ proteins with approximately 24 members, interact with numbers of other cell structures and affects junctional function^[55-58]. Claudins are expressed in a tissue-specific manner and may show distinct functions, for example, in the colon are expressed the claudins-1, 2, 3, 4, 5, 7, and 8; the claudin-2 is a pore-forming TJ protein, but claudins-1 and 4 are barrier tightening proteins^[59-63]. 12-O-tetradecanoylphorbol-13-acetate can increase transepithelial electrical resistance by activating different isoforms of PKC and enhancing the expression of TJ proteins ZO-1, 2, occludin and claudin-1^[64,65]. Ca²⁺/calmodulin-dependent protein kinase II can compromise endothelial barrier function^[66]. Ras-transfected epithelial cells demonstrated compromised barrier function; however, inhibition of the MAPK signaling pathway can restore the morphology of epithelial cells and the TJ assembly. Further, the phosphorylation of tyrosine residues in occludin and ZO-1 may be crucial for the formation of TJ^[67]. cAMP-dependent protein kinases regulate epithelial barrier function by phosphorylation of claudin-3^[68,69].

Generally, at least two relatively independently routes known thus far are responsible for communication between host and external environment through paracellular pathway, both of which can be regulated by protein kinases^[70-72]. The size-selectivity related paracellular pathway is one of the two routes, which facilitates transepithelial passage of different size of molecules, such as lipopolysaccharides^[71,72], and can be regulated by protein kinases, such as MAPKs, Ste20 like proline/alanine rich kinase (SPAK)^[73], PKC^[64,65] and myosin light chain kinase (MLCK)^[74]. Another route, also called charge-selectivity route, is composed of pore-forming proteins claudins^[75-77]. Dysfunction of these two routes, either size-dependent or charge-dependent pathway, may result in the abnormality of overall epithelial TJ, which provides an even more leaky gut. This situation will facilitate the contact of intestinal microorganisms including bacteria, viruses and parasites with the host's immune system, resulting in altered production of inflammatory mediators that contribute to the compromised barrier function.

Mucosal permeability is influenced by many different factors in there distinct ways. Except the mucus layer, microbiota and epithelial cells themselves mentioned above, genetic factors play crucial roles in the regulation of intestinal barrier function^[6]; innate and adaptive immune systems can interfere with epithelial permeability in a dramatic manner^[78]; autonomic nerves, like enteric glial nerve ablation, can perish epithelial permeability to develop fulminant jejunoileitis^[79]. However, barrier dysfunction itself, like in MLCK^[74] and SPAK^[73] gene modified mice, does not necessarily mean that the mice are destined to develop intestinal inflammation, implying formidable compensation in host.

PROTEIN KINASES AND PATHOGENESIS OF IBD

MAPKs

Notably, protein kinases play very crucial roles in many aspects of pathogenesis of IBD, highlighting their emerging roles as potential therapeutic targets against IBD. Besides the NF- κ B pathway, the MAPK signaling pathway is another highlighted pathway involved in many different diseases including IBD^[80]. The activation of MAPK-ERK1/2 phosphorylates the downstream proinflammatory proteins such as cytosolic phospholipase A2 and some transcription factors such as activated proteins, Ets-1, Elk and c-myc. Interestingly, ERK1/2, by a study using an ERK1/2 inhibitor, was found to play an important role in the function of immune cells and other cell types during IBD, by regulating some pro-inflammatory mediators [such as interleukin-1 (IL-1)] related signaling transduction^[81,82], evidenced by their enhanced expression and phosphorylation status during IBD^[83,84]. Furthermore, the "tightening" junction protein claudin-4, which plays an important role in epithelial barrier function, is regulated by protein kinase ERK^[85]. By inducing Akt but blocking p38 signaling, *Lactobacillus* GG prevents cytokine-induced apoptosis of intestinal epithelial cells, indicating p38 and Akt as key mediators of epithelial barrier function^[86,87]. p38 activity is increased significantly in tissues from IBD patients and in mouse models of colitis^[83,84,88], in which inhibition of p38 lowers KC (IL-8) and IL-6 production. A similar result was reported that *heat-killed Lactobacillus brevis* phosphorylates p38 kinase to regulate the expression of proinflammatory cytokines such as TNF- α , and to improve intestinal integrity^[89]. JNK1/2 kinase activity was enhanced in IBD inflamed tissue and blockage of JNK1/2 in experimental colitis reduced the production of proinflammatory cytokines^[84,90,91].

Serine and threonine kinases

SPAK: SPAK is a serine/threonine kinase containing an N-terminal series of proline and alanine repeats (PAPA box) followed by a kinase domain, an NLS, a consensus caspase cleavage motif, and a C-terminal regulatory region^[92]. Colonic SPAK presents as a unique isoform that lacks the PAPA box and F-helix loop in the N-terminus^[93]. The diversity of domains in SPAK might be associated with a variety of biological roles. For example, SPAK was reported to play roles in cell differentiation, transformation and proliferation, and regulation of chloride transport^[94,95]. More importantly, a linkage has been established between SPAK and inflammation. SPAK as an upstream kinase to Na⁺-K⁺-2Cl-co-transporter 1 (NKCC1), can phosphorylate NKCC1 and play an important role in inflammation^[96]. Further, we have demonstrated that SPAK can activate the p38 pathway^[93]. Decreased expression of SPAK contributes to enhanced intestinal barrier, and thus SPAK knockout mice were more tolerant to experimental colitis induced by dextran sodium sulphate (DSS) with

decreased intestinal microorganism translocation into the mucosa and inhibition of the production of inflammatory mediators^[97].

MLCK: MLCK is named after its phosphorylation of MLC to induce contraction of the perijunctional actomyosin ring, and it is indispensable for tumor necrosis factor (TNF) related barrier dysfunction. In turn, TNF can induce the phosphorylation and transcription of MLCK^[98,99]. Constitutive MLCK activation in the intestinal epithelium increases intestinal paracellular permeability and aggravates the severity of colitis in mouse models. However, blockage of MLCK activation can increase significantly the intestinal barrier function and ameliorate DSS-induced colitis^[100].

PKC: PKC has a variety of isoforms that are involved in the pathogenesis of IBD by their effect on the mucus layer^[101], microbiota^[34-37], cell junction^[64,65] and immune system. Specially, PKC θ plays an important role in T cell receptor activation and signaling^[102], and PKC δ is crucial for B cell tolerance^[103,104]. PKC η can control CTLA-4-mediated regulatory T cell (Treg) function^[105]; however, PKC- θ inhibits Treg function, implying its blocking of Treg-mediated suppression. Inhibition of PKC- θ stimulates Treg, resumes compromised Treg function in rheumatoid arthritis patients, and enhances protection against experimental colitis in mice. As a result, PKC- θ mediates negative feedback on Treg cell function^[106].

CONCLUSION

Protein kinases and the related signaling transduction pathways are involved in many physiological and pathological processes such as development, inflammation (for example, intestinal inflammation) and tumorigenesis. In this review, we shed some light on the roles of protein kinases in terms of their effect on IBD-related genetic factors, microbiota, mucus layer, epithelial cell and the tight junction. Further studies are needed to explore the feasibility and application of these signaling pathways in the control of IBD.

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Update on inflammatory bowel disease in patients with primary sclerosing cholangitis

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hold promise for easier, more accurate diagnosis of dysplastic lesions. Chemopreventive measures against colorectal cancer have been sought in these patients. Ursodeoxycholic acid seemed promising at first but subsequent studies yielded conflicting results showing anticarcinogenic effects in low doses (8-15 mg/kg per day) and carcinogenic properties in high doses (15-30 mg/kg per day).

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Key words: Primary sclerosing cholangitis; Inflammatory bowel disease; Ulcerative colitis; Crohn's disease

Abstract

Patients with primary sclerosing cholangitis (PSC) complicated by inflammatory bowel disease (IBD) represent a distinct subset of patients with unique characteristics, which have serious clinical implications. The aim of this literature review was to shed light to the obscure clinical and molecular aspects of the two diseases combined utilizing current data available and putting issues of diagnosis and treatment into perspective. The prevalence of IBD, mainly ulcerative colitis in PSC patients is estimated to be 21%-80%, dependent on screening programs and nationality. PSC-associated colitis is likely to be extensive, characterized by rectal sparing, backwash ileitis, and generally mild symptoms. It is also more likely to progress to colorectal malignancy, making it imperative for clinicians to maintain a high level of suspicion when tackling PSC patients. There is no optimal surveillance strategy but current guidelines advocate that colonoscopy is necessary at the time of PSC diagnosis with annual endoscopic follow-up. Random biopsies have been criticized and a shift towards targeted biopsies using chromoendoscopy, laser endomicroscopy and narrow-band imaging has been noted. Techniques directed towards genetic mutations instead of histological abnormalities

Core tip: Combination of primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD) has recently arisen as a challenging research field. Recent data highlight the specific clinical and genetic traits that differentiate PSC-IBD from the two diseases individually. We reviewed the literature on colorectal neoplastic susceptibility in this subset of patients and the underlying pathogenetic mechanisms. We also emphasize the technological advances that have provided novel diagnostic tools for more accurate detection of dysplastic lesions. Finally, we present current guidelines on follow-up as well as all evidence available as to whether ursodeoxycholic acid should be used prophylactically against colorectal cancer.

Original sources: Tsaitas C, Semertzidou A, Sinakos E. Update on inflammatory bowel disease in patients with primary sclerosing cholangitis. *World J Hepatol* 2014; 6(4): 178-187 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v6/i4/178.htm> DOI: <http://dx.doi.org/10.4254/wjh.v6.i4.178>

INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic progressive disease characterized by inflammation and fibrosis of

medium size and large ducts in the intrahepatic and extrahepatic biliary tree^[1,2]. This disorder results in multifocal intrahepatic and extrahepatic biliary strictures, leading to cholestasis, liver cirrhosis, portal hypertension, and ultimately, premature death from liver failure. It was first reported in the German literature in 1867 by Hoffman, but was described in more detail in the 1920s by two French surgeons, Delbet and Lafourcade. The term sclerosing cholangitis was first used in 1954 by Castleman and later by Schwartz and Dale in their review article^[3]. Its etiology remains largely unknown, although it is strongly believed that autoimmunity is the main culprit. The differential diagnosis of PSC includes congenital diseases (*e.g.*, Caroli disease and choledochal cysts) and secondary cholangiopathy, as observed in patients with collagen vascular diseases (*e.g.*, systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis) and in those with infiltrative diseases (*e.g.*, mediastinal fibrosis, Riedel thyroiditis, eosinophilic cholangitis, and histiocytosis X). Parasitic, fungal, viral or bacterial infections or recurrent cholangitis itself, especially in patients who are immunocompromised, can cause multifocal liver abscesses that lead to a PSC-like appearance of the bile duct. This disease is associated with many cancers including cholangiocarcinoma, gallbladder cancer, hepatocellular carcinoma and colorectal cancer (CRC), thus establishing a link between chronic inflammation and carcinogenesis.

THE PSC-INFLAMMATORY BOWEL DISEASE INTERPLAY

The overwhelming majority of PSC cases have underlying inflammatory bowel disease (IBD). IBD is defined as a chronic condition characterized by immune-mediated inflammation of the gastrointestinal system. The prevalence ranges from 21% to 80%, with the higher rates seen in settings where screening programs are more intense and rectal and sigmoid biopsies are routinely obtained. A geographical variation also exists, with northern European and American societies exhibiting higher rates of PSC-IBD than southern regions and Asia. About 85%-90% of patients with PSC and IBD are comprised of ulcerative colitis (UC) patients and the remainder involves patients with Crohn's colitis or Crohn's ileocolitis^[4]. The association of PSC and Crohn's disease (CD) was first described by Atkinson and Carroll in 1964^[5]. A year later, Smith and Loe described an association between PSC and UC^[6]. Conversely, it has been estimated that PSC occurs in about 5% of UC patients and 3% of CD patients^[7].

IBD may be diagnosed at any time during the course of PSC. Until recently, the diagnosis of IBD more frequently preceded that of PSC, even by several years^[8-11]. Nowadays, there has been a shift in the timing of diagnosis of IBD and PSC. PSC is most commonly diagnosed first, or at least there is a concomitant diagnosis of the two diseases. It is intriguing that *de novo* IBD may present after liver transplantation for PSC^[12], and PSC may present several years after proctocolectomy for IBD. The

altered trend in diagnostic timing can be attributed to two factors. First, the advent of noninvasive imaging techniques such as magnetic resonance cholangiography has enabled early diagnosis of pathological liver biochemistry. Second, the increasing awareness among physicians of the PSC-IBD association has led to early routine endoscopic screening in patients diagnosed with PSC, even in the absence of symptomatic colitis. A study comparing the interval from PSC to IBD diagnosis in 1993-1997 and 2003-2007 showed a decrease from 9 to 7 mo respectively^[13].

The genetic factors for PSC development are still poorly understood. There is an obvious geographic clustering with high prevalence in northern countries compared to Southern Europe and Asia. It has been shown that first-degree relatives of PSC patients have a disease prevalence of 0.7%, representing a nearly 100-fold increased risk of developing PSC compared to that in the general population^[14]. In siblings the prevalence even reaches 1.5%^[15]. Taken together, these epidemiologic data and heritability studies have revealed a strong genetic background for PSC. Genome-wide association studies have shed some light on the subcellular maze of PSC and its overlap with IBD. Human leucocyte antigen (HLA) and non-HLA haplotypes have been identified. The HLA-A1 allele^[16], HLA-C7^[17], major histocompatibility complex class I chain-related A (MICA)*002 and 008/5.1 alleles^[18,19], as well as the tumor necrosis factor (TNF) α promoter -308 A allele^[20] were identified as risk loci for PSC susceptibility. Data from five different European countries (United Kingdom, Italy, Norway, Spain and Sweden) demonstrate that PSC is positively associated with three different HLA class II haplotypes: DRB1*03, DQA1*0501, DQB1*02 (which confers the highest relative risk for PSC development); DRB1*15, DQA1*0102, DQB1*0602; and the DRB1*13, DQA1*0103, DQB1*0603^[21]. However, non-HLA associations have also been confirmed. Of note, MMEL1 and TNFRS14 on chromosome 1p36 encoding a membrane metallo-endopeptidase-like protein of unknown function and a receptor for cytokines and membrane-bound ligands, respectively, have been identified as risk loci for PSC^[22]. The risk of PSC has also been associated with the *FUT2* gene encoding fucosyltransferase^[22], which is an enzyme that regulates expression of the ABO blood group antigens on the surface of epithelial cells. To date, there is scarcity of molecular evidence regarding the shared susceptibility loci between PSC and IBD. A Scandinavian study comparing PSC with UC patients showed distinct HLA associations^[23]. No significant differences were noted between PSC patients with concurrent UC and PSC patients without IBD. This study provides genetic evidence that UC in PSC patients follows a distinct course and demonstrates phenotypic uniqueness compared with UC in isolation. More recently, *REL*, *IL-2* and *CARD9* have been identified as genetic links between the two diseases^[24].

Taking into consideration its associations with HLA haplotypes, autoimmune diseases and the presence of IBD in the majority of PSC patients, immunopathoge-

Table 1 Characteristics of inflammatory bowel disease associated with primary sclerosing cholangitis

Extensive colitis (with right-sided predominance)
Rectal sparing
Backwash ileitis
Mild or quiescent course
Increased risk of colorectal cancer
Increased risk of pouchitis in patients undergoing proctocolectomy with ileal pouch anal anastomosis
Increased risk of peristomal varices in patients undergoing proctocolectomy with ileostomy

netic mechanisms have been sought in PSC pathogenesis. In this regard, two theories have been proposed: the leaky gut hypothesis and the gut lymphocyte homing hypothesis. According to the leaky gut hypothesis, bacteria or bacterial products enter the portal-venous system due to the increased intestinal permeability resulting from inflammation, and translocate to the liver. Bacteria trigger the release of cytokines by Kupffer cells and macrophages in the liver and lead to periductal fibrosis^[8]. The gut lymphocyte homing hypothesis supports the notion that T lymphocytes primed in the inflamed gut may persist as long-lived memory cells, undergo enterohepatic circulation, and trigger portal inflammation in PSC *via* aberrantly expressed adhesion molecules in the liver and gut^[25].

IBD IN PSC: A UNIQUE PHENOTYPIC EXPRESSION

There are many clinical and endoscopic features that differentiate patients with IBD and concomitant PSC and those with IBD in isolation (Table 1). Loftus *et al*^[10] compared 71 patients with PSC who had IBD with a matched group of 142 patients with UC. Among the PSC patients, 86% had UC, 7% had CD, and 7% had indeterminate colitis. The PSC patients more frequently had pancolitis (87% *vs* 54%), rectal sparing (52% *vs* 6%), and backwash ileitis (51% *vs* 7%) than the control group. It is now commonly believed that the colitis associated with PSC is frequently extensive and characterized by rectal sparing and backwash ileitis^[26,27]. These special traits impede the definitive classification of IBD. For instance, the presence of rectal sparing or ileitis may be misinterpreted for CD or indeterminate colitis, rather than UC. In addition, PSC-associated colitis runs a milder, quiescent course, sometimes with absent clinical manifestations, thus delaying diagnosis^[28]. Another intriguing trait in PSC-IBD patients is the higher rate of colorectal neoplasia, which tends to be proximal, is diagnosed at a later stage and has a worse prognosis. The colorectal neoplastic potential in these patients will be discussed later.

Of note, PSC patients who have an ileal pouch anal anastomosis (IPAA) after colectomy have an increased risk of pouchitis compared to patients with UC without PSC^[29,30]. The underlying mechanism for this complica-

tion remains obscure. There is also one report suggesting that patients with PSC and IPAA run an increased risk of development of dysplasia in the ileal pouch mucosa compared with UC patients without PSC, and that these patients consequently should be under intensive surveillance^[31]. However, more studies are required to substantiate these claims. Interestingly, it has been suggested that PSC-IBD patients undergoing proctocolectomy with ileostomy develop peristomal varices more frequently than IBD patients without evidence of hepatobiliary disease^[32]. Bleeding from these often is recurrent and is challenging to treat. This complication can be controlled with a portosystemic shunt or transjugular intrahepatic portosystemic shunt, but liver transplantation may be considered.

PSC in patients with IBD does not seem to run a different course when compared to patients without IBD. Nevertheless, one study has demonstrated that PSC in patients with concomitant IBD has a predilection for men, is more likely to manifest itself for the first time with abnormal liver biochemistry, and has intrahepatic and extrahepatic biliary tree strictures^[33]. Proctocolectomy, as a surgical treatment for UC, has no effect on liver function tests, histology or survival of patients with PSC^[34].

COLORECTAL NEOPLASTIC POTENTIAL IN PSC-IBD PATIENTS

The increased occurrence of CRC in IBD patients has been well documented since 1925, when it was first described by Crohn and Rosenberg^[35]. The cancer risk involves both UC and CD^[36-39], and has been linked with prolonged duration and extent of disease, associated PSC and active inflammation^[40,41]. Data on the relative risk of CRC in IBD are not in agreement in different studies. The cumulative risk varies from 1.4% after 18 years^[42] to 34% after 25 years from onset of disease^[43]. Some studies even advocate that the risk is not increased at all^[44].

Recent investigations have unveiled another relationship, that of PSC-IBD patients and CRC. The concept that PSC is associated with an increased risk of colorectal neoplasia in patients with UC was proposed by Broomé *et al*^[45] in 1992. In a study of 17 patients with UC who were found to have dysplasia, carcinoma, and/or DNA aneuploidy, 28% had coexistent PSC. This led to the hypothesis that PSC is an independent risk factor for the development of colorectal neoplasia in patients with existing UC. There has been a wealth of studies since then reporting the connection of PSC-IBD and CRC, particularly highlighting the compounding neoplastic risk when the two disorders coexist as opposed to patients with IBD alone^[46-53] (Table 2). Soetikno *et al*^[54] performed a meta-analysis of 11 studies and described an odds ratio (OR) of 4.79 (95%CI: 2.89-5.76) when comparing patients with UC and PSC to UC patients without PSC. However, two studies (both from the Mayo Clinic but using different groups of patients) have yielded contradictory results rejecting the hy-

Table 2 Summary of studies evaluating primary sclerosing cholangitis as a risk factor for colorectal neoplasia in chronic ulcerative colitis

Ref.	UC case group (No)	Centre	End point (No)	Matched controls	Colectomy rate	Is PSC a risk factor?
Broomé <i>et al</i> ^[45]	Dys (17)	Hudding, Sweden	PSC (5)	Yes	0%	Yes
D'Haens <i>et al</i> ^[46]	Dys (29)	Chicago, United States	Cholestasis/PSC (10)	Yes	0%	Yes
Broomé <i>et al</i> ^[47]	PSC (40)	Hudding, Sweden	CRC/Dys (15)	Yes	30%	Yes
Brentnall <i>et al</i> ^[48]	PSC (20)	Seattle, United States	Dys (9)	No	0%	Yes
Leidenius <i>et al</i> ^[49]	PSC (45)	Helsinki, Finland	CRC/Dys (13)	Yes	29%	Yes
Marchesa <i>et al</i> ^[50]	PSC (27)	Cleveland, United States	CRC (4)/Dys (14)	Yes	All postop	Yes
Shetty <i>et al</i> ^[51]	PSC (132)	Cleveland, United States	CRC (17)/Dys (16)	No	0%	Yes
Loftus <i>et al</i> ^[52]	PSC (143)	Mayo Clinic, United States	CRC (8)	No	37%	No
Nuako <i>et al</i> ^[53]	CRC (171)	Mayo Clinic, United States	PSC (30)	Yes	14%	No

CRC: Colorectal cancer; CRN: Colorectal neoplasia; Dys: Dysplasia; PSC: Primary sclerosing cholangitis.

pothesis that there is an increased risk for CRC in PSC-IBD patients^[52,53]. In general, the increased neoplastic potential in PSC-IBD patients could be ascribed to late diagnosis due to the subclinical course of colitis and the conservative treatment of mild flare-ups as opposed to colectomy, thereby increasing the duration and extent of colitis.

CRCs associated with PSC display a number of characteristics. They appear to have a more proximal localization with up to 76% right-sided distribution. A full colonoscopy is therefore mandatory for surveillance purposes. CRCs in this subset of patients are diagnosed at a more advanced stage and tend to be fatal. In a recent study, PSC patients with IBD and CRC were found to be younger at onset of IBD than patients who had IBD and CRC without PSC (19 *vs* 29 years; $P = 0.04$). The time interval from onset of colitis until diagnosis of CRC was, however, similar in the two groups (17 *vs* 20 years; $P = 0.02$)^[55].

PATHOGENETIC MECHANISMS OF CRC IN PSC

The mechanisms underlying the pathogenesis of CRC in IBD patients have been rigorously investigated and many differences in comparison with sporadic CRC have been addressed^[56,57]. Even though IBD-CRC usually follows a dysplasia-cancer pattern, as in sporadic cancer, molecular and genetic events seem to occur in an unconventional sequence. Alterations to the *p53* tumor suppressor gene occur earlier in colitis-related CRC^[58], whereas adenomatous polyposis coli (*APC*) gene alteration is usually a later event^[59]. The reverse applies to sporadic CRC. It is also noteworthy that *p53* mutations can be present in nondysplastic mucosa in IBD-CRC, but only in dysplastic areas in sporadic cancer^[60]. Another noteworthy difference is that low-grade dysplasia is often in flat lesions in CRC-IBD, which are difficult to detect endoscopically, whereas such dysplasia occurs within raised polyps in sporadic cancer. The role of microsatellite instability, hypermethylation, chromosomal instability, interleukin (IL)-23/IL-17 signaling and E-cadherin (CDH1) has been addressed in studies but is not yet fully understood^[61-64]. Polymorphisms of the mismatch repair genes *MLH1* and *MSH2*

have been incriminated for the pathogenesis of IBD and related malignancy^[65,66].

The direct impact of PSC on colorectal carcinogenesis has not yet been delineated. Another theory highlights the significance of the cholestasis-associated secondary bile salt pool in the colon^[67]. Bile acids such as deoxycholic acid and lithocholic acid (LCA) are thought to contribute to tumorigenesis through disruption of the balance between colorectal crypt cell proliferation, differentiation and apoptosis^[68-71]. Mucosa in carcinoma displayed an increased frequency of bile acid receptors compared with normal tissue^[72]. A higher fecal bile acid concentration was found in patients with UC who developed neoplasia compared with those without UC^[73]. Folate deficiency has also been implicated in the pathogenesis of CRC in IBD patients. Folate deficiency arises from sulfasalazine use to treat UC, which is a competitive inhibitor of folate absorption. Folate supplementation was associated with a 62% reduction in the incidence of neoplasia in patients with pancolonic UC compared with placebo^[74]. This theory, however, contradicts epidemiological reports according to which maintenance therapy in UC reduces risk of carcinogenesis^[75].

SURVEILLANCE RECOMMENDATIONS

Periodic surveillance colonoscopy is the milestone of cancer prevention in IBD^[76,77]. PSC in combination with IBD further enhances the risk for CRC as described above and necessitates increased alertness. That along with the fact that IBD in PSC patients usually follows an asymptomatic, subclinical course raises the need for routine colonoscopy at the time of PSC diagnosis, which should be repeated on an annual basis^[78]. However, a recent study by Imam *et al*^[79] showed a low risk of colonic neoplasia in young patients with a combined diagnosis of PSC and IBD, with an estimated prevalence of 1.3% and an incidence of 0.4% per year. This finding raises the question whether annual surveillance is unnecessary in this selected group of patients.

Three categories of dysplasia have been identified according to the IBD Dysplasia Morphology Study Group^[80]: (1) negative for dysplasia; (2) indefinite for dysplasia; and (3) positive for dysplasia, which is further subdivided into low-grade dysplasia (LGD) and high-grade

dysplasia (HGD). Each of these categories necessitates a different approach. A finding of indefinite dysplasia dictates a repeat colonoscopy in 3-6 mo. The management of LGD is debatable with no clear evidence of optimal approach. St Mark's Hospital^[81] demonstrated a 54% cumulative probability of LGD progressing to HGD or CRC. Mayo Clinic reported a 33% 5-year progression^[82], while other studies described an even lower rate^[83,84]. Thus, in the case of LGD different options should be discussed with patients and informed consent for conservative or operative management should be obtained. Patients with multifocal flat LGD in one screening or unifocal LGD in more than one screening should prompt prophylactic total proctocolectomy. HGD, however, needs unquestionable referral for total proctocolectomy due to the increased risk of concurrent or subsequent malignancy^[85].

There has been increased skepticism in the medical community about the random biopsies used for surveillance purposes. New methods enable targeted biopsies to be obtained from identifiable lesions. Chromoendoscopy, confocal laser endomicroscopy and narrow-band imaging (NBI) are new promising techniques that are likely to replace old-fashioned random biopsies that have proven their inadequacy in many studies^[86,87]. Chromoendoscopy uses the application of indigo carmine or methylene blue to stain dysplastic areas on the colonic mucosa. Hurlstone *et al*^[88] examined 700 patients in a prospective case-control trial and diagnosed 69 dysplastic lesions with chromoendoscopy and only 24 with random biopsies ($P < 0.001$)^[88]. Confocal laser endomicroscopy enables the histological visualization of the mucosa in real time. It is easily inferred that this technique requires specialized training for histological interpretation. A randomized controlled trial was conducted by Kiesslich *et al*^[89] showing an increase of 4.75 times in yield of neoplasia when using endomicroscopy ($P = 0.005$). NBI uses optical fibers to enable a clear visualization of vessels, pit pattern and soft tissue structures. A study showed that NBI cannot be recommended as a chromoendoscopy substitute because it detected fewer lesions than chromoendoscopy in chronic colitis, although most were not dysplastic^[90].

Newer techniques that target genetic alterations rather than histological abnormalities have been proposed to increase detection efficacy. Deletions and point mutations of tumor-suppressor genes such as *p53*, *Rb*, *APC*, *mc* and the Sialosyl-Tn antigen have been found in dysplastic lesions and could be a useful tool for early diagnosis^[91,92]. DNA evaluation by flow cytometry could reveal aneuploidy and predict suspicious areas likely to progress to CRC. The main drawback is that aneuploidy is not always a prerequisite for cancer occurrence and its presence does not always lead to malignancy.

CHEMOPREVENTION

Many studies have investigated the potential protective effects of different drug agents against malignancy in patients with UC. Pinczowski *et al*^[93] was the first to report

that CRC risk is diminished by therapy with 5-aminosalicylic acid (5-ASA) in 1994. Ever since, studies have failed to demonstrate a clear relationship between 5-ASA therapy and CRC, rendering its potential protective effect presumptive rather than definitive. Azathioprine and mercaptopurine have not been shown to have a beneficial effect with regards to CRC in IBD^[85]. Likewise, research has yielded inconclusive results regarding the use of corticosteroids, nonsteroidal anti-inflammatory drugs or folates for chemopreventive purposes.

Ursodeoxycholic acid (UDCA) is a drug commonly used in PSC patients due to its safe profile and favorable effects on the biochemical parameters of the disease. *In vitro* and animal studies have revealed a chemoprophylactic effect of UDCA. UDCA appears to arrest proliferation of colon cancer cell lines *in vitro*^[94]. CRC induced by N-methylnitrosourea^[95] and azoxymethane^[96,97] in rats appeared to respond to UDCA therapy with a decrease in size. UDCA also decreased fecal concentrations of deoxycholic acid in animals, suggesting a potential protective effect through control of bile acid concentration in colon^[98]. Several molecular mechanisms have been proposed to explain the chemoprophylactic effect of UDCA, including downregulation of cyclo-oxygenase-2 expression^[97], prevention of carcinogen-induced changes in protein kinase C isoforms^[99], suppression of epidermal growth factor receptor^[100], cell cycle modulation by inhibiting the expression of cyclin D1 and promoting that of E-cadherin^[101], and stabilization of mitochondrial membranes against damaging free radicals^[102]. These results triggered a number of investigations in humans in order to shed light on the real effect of UDCA. Two studies have confirmed the protective effect of the drug. Tung *et al*^[103] conducted a retrospective review of 59 patients showing a reduced OR of 0.18 (95%CI: 0.05-0.61) of colonic dysplasia after ursodiol use. Pardi *et al*^[104] also performed a randomized placebo controlled study evaluating the effect of UDCA in the subgroup of UC patients with concomitant PSC. Patients who received a low dose of UDCA (13-15 mg/kg per day) showed a relative risk of 0.26 for developing CRC or dysplasia. Wolf *et al*^[105] showed in a retrospective study of 120 patients that there was no reduction of CRC or dysplasia in the UDCA group. A hallmark study by Eaton *et al*^[106] has recently reversed the long-standing conviction that UDCA has a place in CRC prevention in PSC-IBD patients. Using high doses of UDCA (28-30 mg/kg per day), they showed an increased risk of CRC in the UDCA group. The majority of patients developed colorectal neoplasia after > 2 years of use. This association remained significant after adjusting for smoking history and UC duration. High-dose UDCA also resulted in an increased risk of liver transplantation and/or death^[107]. The discrepancy between different studies can be attributed to their inherent limitations and their failure to adjust for confounding factors such as age at onset of colitis, extent of colitis, family history of CRC, cigarette smoking, use of other drugs such as 5-ASA and folate, and use of the same criteria for dysplasia classification.

There has been speculation with regard to mechanisms underlying the toxic and carcinogenic UDCA properties. The most prevalent theory implicates the alteration of colonic bile acid milieu when high doses are used. An increase in serum UDCA and LCA levels in the treatment group has been reported^[108]. That combined with results from *in vitro* studies stating that bile acids stimulate cell invasion in a dose-dependent fashion and reduce apoptosis could possibly provide a plausible explanation of the differing effects when low and high UDCA doses are used^[109-111]. It is therefore prudent to recommend UDCA chemoprevention only to a high-risk subset of patients, including those with a personal or family history of CRC, and those with long-standing extensive colitis. This rationale has been incorporated to recent European guidelines^[112].

In conclusion, PSC-IBD patients represent an important public health concern. Significant steps have been made towards the elucidation of the pathogenetic mechanisms underlying this complex disease. HLA and non-HLA susceptibility genes have been thoroughly studied and proven their association with PSC-IBD. Further investigations are warranted to reveal PSC- and IBD-specific genes and clarify their real impact on the disease. Genome-wide association studies could be invaluable in this direction but are severely undermined by the rarity of the disease and therefore the limited number of PSC patients that can be recruited. In terms of diagnosis, biomarkers currently in use are liver function tests and histology. A couple of new methods have been introduced to facilitate the evaluation of PSC patients. Fibroscan and a breath test assessing the elasticity and metabolic capacity of the liver respectively have paved the way for rapid, non-invasive diagnosis. Their diagnostic accuracy in PSC, however, remains under scrutiny.

CRC is a well-established risk for PSC-IBD patients. Aggressive colonoscopic surveillance is therefore imperative, even in those who have undergone liver transplantation^[113]. In an attempt to relieve the socioeconomic and medical burden that PSC-IBD poses, many studies have explored potential pharmaceutical agents that may retard disease progression and protect against colorectal neoplasia. Antibiotics, immunomodulators, UDCA and antifibrotic agents have attracted the attention of researchers but their full potential has not yet been unraveled. Recent meta-analyses have demonstrated that UDCA in low to medium doses seems to have a chemoprophylactic effect, whereas high doses are carcinogenic^[114,115]. Further investigations are required to test the efficacy of existing drug agents and promote the development of new ones. Understanding and harnessing molecular events seems a pivotal step towards this direction.

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Colorectal cancer surveillance in inflammatory bowel disease: A critical analysis

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Abstract

Colonoscopic surveillance is advocated in patients with inflammatory bowel disease (IBD) for detection of dysplasia. There are many issues regarding surveillance in IBD: the risk of colorectal cancer seems to be decreasing in the majority of recently published studies, necessitating revisions of surveillance strategy; surveillance guidelines are not based on concrete evidence; commencement and frequency of surveillance, cost-effectiveness and adherence to surveillance have been issues that are only partly answered. The traditional technique of random biopsy is neither evidence-based nor easy to practice. Therefore, highlighting abnormal areas with newer technology and biopsy from these areas are the way forward. Of the newer technology, digital mucosal enhancement, such as high-definition white light endoscopy and chromoendoscopy (with magnification) have been incorporated in guidelines. Dyeless chromoendoscopy (narrow band imaging) has not yet shown potential, whereas some forms of digital chromoendoscopy (i-Scan more than Fujinon intelligent color enhancement) have shown promise for colonoscopic surveillance in IBD. Other techniques

such as autofluorescence imaging, endomicroscopy and endocytoscopy need further evidence. Surveillance with genetic markers (tissue, serum or stool) is at an early stage. This article discusses changing epidemiology of colorectal cancer development in IBD and critically evaluates issues regarding colonoscopic surveillance in IBD.

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Key words: Advanced imaging; Chromoendoscopy; Colorectal cancer; Colorectal cancer surveillance; Inflammatory bowel disease

Core tip: There is an increase in the risk of colorectal cancer in patients suffering from inflammatory bowel disease. Recent studies have suggested that this risk may be decreasing. In view of the risk, colonoscopic surveillance is recommended in order to detect cancer early. Instead of using previous methods of colonoscopy and random biopsy, newer technology such as chromoendoscopy and biopsy from abnormal mucosa is preferable.

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CHANGING EPIDEMIOLOGY OF COLORECTAL CANCER IN INFLAMMATORY BOWEL DISEASE

The risk of colorectal cancer (CRC) in patients with inflammatory bowel disease (IBD) was recognized as far back as 1925 for ulcerative colitis (UC) and 1948 for

Crohn's disease (CD)^[1,2]. In the second half of the last century, attempts were made to quantify the actual risk of CRC in this population. Earlier studies, mainly in UC patients, tended to overestimate the risk, with cumulative cancer rates reportedly ranging from 16% to 43%^[3-7]. A widely cited meta-analysis of 116 studies with age stratified data by Eaden *et al*^[8] in 2001 estimated CRC risk as 2% at 10 years, 8% at 20 years and 18% at 30 years. In 2006, two landmark studies suggested a decreasing trend of CRC in IBD. Jess *et al*^[9] reported a population-based estimate of CRC in IBD from Olmsted County, Minnesota, US. They reported that the risk of CRC was not increased in UC as compared to the general population [standardized incidence rate (SIR) 1.1; 95%CI: 0.4-2.4], but the risk of CRC was increased in CD (SIR 1.9; 95%CI: 0.7-4.1); the cumulative cancer risk was 2% at 20 years. The other study by Rutter *et al*^[10] from St. Marks Hospital, United Kingdom, reported a CRC risk of 2.5% at 20 years, 7.6% at 30 years and 10.8% at 40 years, which was less than that reported by Eaden *et al*^[8]. The lower risk of CRC was confined to a location proximal to the splenic flexure, but not at other locations. There were two subsequent meta-analyses. The study by Jess *et al*^[11] in 2012 shortlisted eight population-based studies from 1958 to 2004 and reported a risk of 1.6% in patients with UC over 14 years of follow-up; UC increased the risk of CRC 2.4-fold (pooled SIR 2.4, range: 1.05-3.1; 95%CI: 2.1-2.7). The meta-analysis by Lutgens *et al*^[12] also shortlisted eight studies from 1988 to 2009 and reported that the risk of CRC was increased in IBD, but was not as high as reported in earlier studies; the pooled SIR was 1.7 (CI: 1.2-2.2). Two recent studies came to different conclusions. In a population-based study from Denmark, Jess *et al*^[13] suggested that the risk of colon cancer in UC is not as high as previously reported and in fact may not differ from the general population. To the contrary, Herrinton *et al*^[14] showed that the risk of CRC in UC is 60% higher than in age- and gender-matched cohorts of people without IBD from California, and the risk remained the same throughout the study period of 14.5 years. Studies from Asia on CRC in UC are few, and report that the likelihood ranges from 0.87% to 1.8% in general, and can be as high as 13.5% in patients with extensive colitis^[15-21].

The risk of CRC in CD was initially underestimated because of failure to evaluate cases of colitis as a separate risk group and to account for the effect of early colectomy. It is now established that patients with colonic or ileocolonic CD have an increased risk of CRC compared to the general population. A meta-analysis of 12 population- and hospital-based studies published in 2006 confirmed an overall relative risk (RR) of 2.5 (95%CI: 1.3-4.7) and an RR of 4.5 in those with colonic CD (95%CI: 1.3-14.7)^[13]. The risk for those with ileal disease only was the same as the general population. Regardless of disease distribution, the cumulative risk of CRC was 2.9% after 10 years, 5.6% after 20 years, and 8.3% after 30 years of disease.

Thus, the risk of CRC is increased in IBD, though there is variation due to various factors such as referral

center bias, population- or hospital-based data, and small numbers of patients. Prevalence rates of CRC in UC vary from 0.7% to 3.3%^[9,22-28] and the cumulative risk is 1%, 2% and 5% for 10, 20, and > 20 years of disease duration, respectively, and a pooled SIR of 1.7 in all patients with IBD in population-based studies^[13]. Table 1 summarizes the risk of CRC in IBD in various population groups. The surveillance strategy needs to take into account this decreasing risk of CRC in IBD.

GUIDELINES FOR SURVEILLANCE OF CRC IN IBD

Guidelines by various societies suggest that surveillance for CRC should begin after 8-10 years of disease duration. The guidelines include those from the American Gastroenterological Association (AGA; 2004 and 2010), Association of Coloproctology for Great Britain and Ireland (2004 and 2010), British Society of Gastroenterology (BSG; 2002 and 2010), National Institute for Health and Clinical Excellence (2011), European Crohn's and Colitis Organization (2013), and Australian (2011) and Austrian societies^[29-34]. Table 2 summarizes the guidelines with changes over time. The salient features of the guidelines include that surveillance is advised 8-10 years after the onset of symptoms, irrespective of the extent (surveillance is not advised in patients with proctitis and proctosigmoiditis). The frequency of surveillance varies amongst the guidelines: AGA guidelines initially suggest surveillances every 1-2 years, and if two examinations are negative, then every 1-3 years up to the end of the second decade, after which the surveillance is again every 1-2 years. In the BSG guidelines, the frequency of surveillance depends upon the risk. Lower risk requires surveillance every 5 years, which includes extensive colitis with no endoscopic or histologic inflammation, left-sided colitis or crohn's colitis (involving < 50% colon). Surveillance every three years is recommended for intermediate risk, which includes extensive colitis with mild active endoscopic, histologic inflammation, post inflammatory polyps, or family history of CRC in first-degree relatives over 50 years of age. Yearly surveillance is needed for those with higher risk, including extensive colitis with moderate or severe endoscopic or histologic inflammation, stricture or dysplasia in the past five years where patients have declined surgery, primary sclerosing cholangitis, or family history of CRC in a first-degree relative less than 50 years of age (surveillance yearly). The method of surveillance also varies, from random biopsy every 10 cm, which is still advocated, though the preferred method is to use chromoendoscopy and magnification and take biopsies from the abnormal areas.

Comparison of American and British guidelines

Mooiweer *et al*^[35] from the Netherlands compared the American and British guidelines in a retrospective study of 1018 patients. They concluded that BSG surveillance intervals offer the advantage of a lower colonoscopic

Table 1 Risk of colorectal cancer in inflammatory bowel disease

Ref.	Type of study	Risk in UC	Risk in CD	Odds ratio (95%CI)	Comments
Eaden <i>et al</i> ^[8] 2001	Meta-analysis 116 studies; 41 mentioned duration of UC	3.7%		NA	2% at 10 yr, 8% at 20 yr, 18% at 30 yr
Jess <i>et al</i> ^[9] 2006	Population-based	6/378 (1.6%)	6/314 (1.9%)	SIR UC: 1.1 (0.4-2.4) CD: 1.9 (0.7-4.1)	Cumulative cancer risk 2% at 20 yr
Rutter <i>et al</i> ^[10] 2006	Hospital-based retrospective	3/600 (0.5%)	NA	NA	2.5% at 20 yr, 7.6% at 30 yr, 10.8% at 40 yr
Jess <i>et al</i> ^[11] 2012	Meta-analysis of 8 population-based (1958-2004)	1.6% (14 yr follow-up)	NA	Pooled SIR: 2.4 (2.1-2.7)	Risk of in patients with UC over
Lutgens <i>et al</i> ^[12] 2013	Meta-analysis (1988-2009)			IBD pooled SIR Population based: 1.7 (1.2-2.2) Referral based: 5.3 (2.8-7.8) RR for CRC- UC 1979-1988: 1.34 (1.13-1.58) 1989-1998: 1.09 (0.9-1.33) 1999-2008: 0.57 (0.41-0.80) RR for CRC in CD: 0.85 (0.67-1.07), which did not change over time	CRC risk in UC reduced over three decades and comparable to general population; CD no change
Jess <i>et al</i> ^[13] 2012	Population-based			UC: 1.6 (1.3-2.0) CD: 1.6 (1.2-2.0)	CRC risk in UC and CD 60% higher than population
Herrinton <i>et al</i> ^[14] 2012	Hospital-based	UC 53 /10895 CD 29/5603			
Asian studies					
Gilat <i>et al</i> ^[15] 1988	Population-based (central Israel)		NA		CRC risk in UC: 0.2% at 10 yr, 5.5% at 20 yr, 13.5% at 30 yr
Kochhar <i>et al</i> ^[16] 1992	Hospital-based (India)	UC 1.8%	NA		
Venkataraman <i>et al</i> ^[17] 2005	Hospital-based (India)	UC 0.94%			
Kim <i>et al</i> ^[19] 2009	Population-based (South Korea)	UC 0.50%			
Kekilli <i>et al</i> ^[20] 2010	Hospital-based (Turkey)	UC 1.10%			
Gong <i>et al</i> ^[21] 2012	Hospital-based (China)	UC 0.87%			

CD: Crohn's disease; FH: Family history; IBD: Inflammatory bowel disease; NA: Not applicable; PSC: Primary sclerosing cholangitis; RR: Relative risk; SIR: Standardized incidence rate; UC: Ulcerative colitis.

workload (421 colonoscopies as per BSG guidelines and 541 colonoscopies as per AGA guidelines). However, the risk stratification of the AGA appears superior in distinguishing patients at higher risk of colitis-associated neoplasia (AGA: 5.3 and 20.3% in low and high risk groups, respectively; BSG: 3.6, 6.9 and 10.8% in low, intermediate and high-risk groups, respectively).

ISSUES WITH SURVEILLANCE

Is surveillance really necessary?

Most of the above guidelines suggest that surveillance is recommended based on the high risk of CRC in IBD^[8]. However, a recent study by Jess *et al*^[13] suggested that the incidence of CRC in UC in a Danish population decreased over 30 years (1979-2008), and the risk was not different from the general population during the period of 1999-2008 (RR 0.8). There is no systematic surveillance in Denmark. In their population-based study from patients in the US, Jess *et al*^[9] reported no overall increase in CRC in all UC patients but only in patients with extensive colitis. A Danish article commented that, based on Danish epidemiologic data, the American and British recommendations were dubious and surveillance may be recommended in patients with extensive, uncontrolled

inflammation and patients with primary sclerosing cholangitis, and not on the disease duration^[56]. Thus, although surveillance is recommended by all societies, routine surveillance may not be beneficial and surveillance strategies should be reviewed due to the reduction in risk of CRC in UC.

When should surveillance begin?

The guidelines suggest that surveillance for CRC should begin after 8-10 years of disease duration. However, if these recommendations are followed, CRC is likely to be missed. In the study by Gilat *et al*^[15], 2/26 patients who developed CRC in UC had a disease duration less than ten years (six and nine years)^[16]. The cumulative risk of CRC in the first decade was 1.15% in the study by Gong *et al*^[21], and 1.6% in the meta-analysis by Eaden *et al*^[8]. Lutgens *et al*^[37] reported that 15% of their patients with UC developed CRC before the recommended surveillance period. Kocher *et al*^[16] reported that 2/8 patients developed CRC at seven and eight years of disease duration. Thus, we are faced with a dilemma: on one hand, the incidence seems to be decreasing, whereas on the other hand, we are likely to miss about 15%-20% of patients who develop CRC before the recommended commencement of surveillance.

Table 2 Guidelines of various societies on surveillance for colorectal cancer in ulcerative colitis

Society	Year	Beginning of surveillance	Frequency	Technique	Biopsy protocol	Risk	Change
BSG	2002	All patients have colonoscopy screening at 8-10 yr; surveillance begins 8-10 yr after onset for pancolitis, 15-20 yr for left-sided colitis	Decrease in surveillance interval with increase in disease duration for pancolitis: Every 3 yr: 2 nd decade Every 2 yr: 3 rd decade Every 1 yr: 4 th decade	Nil	2-4 random biopsies every 10 cm from the entire colon	Patients with PSC, including those with OLT, should have annual screening	
AGA	2004	8-10 yr	Every 1-2 yr	Nil			
ACG	2004	8-10 yr	Every 1-2 yr	Nil			
ECCO	2008	8 yr for pancolitis, 15 yr for left-sided colitis	Every 2 yr: 1 st two decades Every 1 yr: 3 rd decade	CE			
BSG	2010	10 yr	Based on extent of disease, endoscopic and histologic activity, FH of CRC, presence of PSC, pseudopolyps, stricture, dysplasia on biopsy: Every 3 yr: low risk Every 2 yr: intermediate risk Every 1 yr: high risk	CE	Random biopsies every 10 cm and biopsies from raised/suspicious areas on CE	Patients with PSC, including those with OLT, should have annual screening	If dysplastic polyp within area of inflammation can be removed entirely, colectomy is not necessary
AGA	2010	8-10 yr	Every 1-2 yr If two examinations are negative, then every 1-3 yr up to 20 yr, then every 1-2/yr	CE		Patients with PSC, including those with OLT, should have annual screening	
NICE	2011	10 yr	As per BSG 2010 guidelines	CE			
Australian	2011	8-10 yr	As per BSG 2010 guidelines	CE			
ECCO	2013	6-8 yr, 8-10 yr	Same as BSG	CE			

ACG: Association of Coloproctology for Great Britain and Ireland; AGA: American Gastroenterological Association; BSG: British Society of Gastroenterology; CE: Chromoendoscopy; CRC: Colorectal cancer; ECCO: European Crohn's and Colitis Organization; FH: Family history; NICE: National Institute for Health and Clinical Excellence; OLT: Orthotopic liver transplantation; PSC: Primary sclerosing cholangitis.

ROLE OF NEWER MODALITIES FOR SURVEILLANCE IN IBD

There are clear lapses in the present form of colonoscopic surveillance. The random biopsy technique is not very useful for detecting dysplasia. In a retrospective analysis of 11772 biopsies in 466 colonoscopies in 167 patients over ten years, this technique had a much lower yield of dysplasia as compared to targeted biopsies and did not significantly change the management^[38,39]. Two retrospective studies have shown that dysplasia in IBD is macroscopically visible in 72%-77% of patients^[40,41]. Based on a single retrospective study, high-definition endoscopy is three times more likely to detect dysplastic lesions as compared to standard-definition endoscopy^[42].

Chromoendoscopy and magnification chromoendoscopy have been used for the detection of dysplastic lesions that are likely to be missed by white light endoscopy. A meta-analysis of six studies showed that the yield with chromoendoscopy was 7% greater than that of white light endoscopy, and the pooled increase in targeted dysplasia detection of chromoendoscopy over white light endoscopy was 44% (95%CI: 28.6-59.1)^[43]. The difference in detection of flat dysplastic lesions was 27% (95%CI: 11.2-41.9). Chromoendoscopy has been incorporated in the recent guidelines.

Dyeless chromoendoscopy includes compound-band imaging and narrow-band imaging, which fails to detect dysplasia in patients with IBD and has not been recom-

mended for surveillance in its present form^[39]. Digital chromoendoscopy includes i-Scan and Fuji intelligent chromoendoscopy, which have not been studied in clinical trials in IBD patients to detect dysplasias. They have been used to detect adenomas in surveillance programs in CRC in a non-IBD population, where only i-Scan demonstrated some positive results^[39]. Studies using autofluorescence imaging have shown that it is a sensitive modality to detect dysplastic lesions in IBD^[44]. Confocal laser endomicroscopy and endocytoscopy allow for magnification of up to 1390-fold. Confocal laser endomicroscopy detects more dysplasia than white light and chromoendoscopy, but requires special training and takes twice as much time^[45,46]. Table 3 summarizes the important features of these modalities.

Although the pathogenesis of CRC in IBD differs from sporadic CRC, polyposis syndromes and hereditary non-polyposis colon cancers, the pathways include chromosomal instability, microsatellite instability and CpG island methylation pathways. Tissue-based markers, such as aneuploidy, p53, and microsatellite instability, are associated with the development of dysplasia or CRC^[30]. They cannot be included in the guidelines for surveillance for CRC in IBD at present.

NON-COLONOSCOPIC APPROACHES FOR CANCER SURVEILLANCE IN IBD

Non-colonoscopy techniques that are noninvasive are

Table 3 Endoscopic dysplasia-detection modalities in patients with inflammatory bowel disease and recommendations for use^[39]

	Demonstrated accuracy in IBD	Supporting evidence in IBD	Incorporated into guidelines	Practicality of use in practice	Should be used in 2013?
Random biopsy	-	-	+	±	±
HD WLE	+	±	+	+	+
Chromoendoscopy	+	+	+	+	+
NBI	-	-	-	±	-
FICE	NA	NA	-	±	-
i-Scan	NA	NA	-	±	-
AFI	+	+	-	-	-

AFI: Auto-fluorescence imaging; HD WLE: High-definition white light endoscopy; FICE: Fuji intelligent chromoendoscopy; IBD: Inflammatory bowel disease; NA: Not available; NBI: Narrow-band imaging. Reproduced with permission^[39].

more appealing to patients than the repeated invasive colonoscopic approach, with the potential to reduce the high cost associated with surveillance. Stool examination has been used for surveillance for sporadic CRC and stool DNA testing has recently been incorporated^[47]. Studies by Kisiel and others suggest that stool DNA testing is feasible to detect CRC in patients with IBD^[48,49]. Although this approach is not recommended for surveillance at present, it has the potential to radically change the approach to surveillance.

IS SURVEILLANCE EFFECTIVE? DOES SURVEILLANCE SAVE LIVES? IS IT COST-EFFECTIVE?

Multiple case series and case control studies have suggested that surveillance leads to improvement in survival in UC, which was not supported by a Cochrane systematic review^[50-58]. The data from the Cochrane analysis suggests that there is no clear evidence that surveillance colonoscopy prolongs survival in patients with extensive colitis. In patients undergoing surveillance, CRC is detected at an earlier stage, which may lead to a better prognosis (which may actually be due to lead-time bias). Surveillance may be effective in reducing the risk of death and it may be cost-effective. These findings have to be taken with the facts that these pivotal studies were in the 1990s, and the Cochrane analysis was in 2004. Studies showing a reduction in CRC have been published after these studies and this analysis may not hold true in the situation with reduced risk of CRC in UC. Surveillance is advocated in CD but there is no data to support it^[30].

ADHERENCE OF PHYSICIANS AND PATIENTS TO SURVEILLANCE COLONOSCOPY

There is a wide variation in conducting colonoscopic surveillance by gastroenterologists. Eaden *et al.*^[59] reported that all British gastroenterologists perform colonoscopic surveillance in pancolitis, but only 24% practiced surveillance in left-sided colitis, and only 2% took more than 20 biopsies. In a survey from the Netherlands, 95% of gas-

troenterologists performed colonoscopic surveillance in UC and 65% in CD; a majority (73%) of gastroenterologists took fewer than 30 biopsies, and only 27% followed AGA guidelines^[60]. From this and similar data, it is clear that the concept of colonoscopic surveillance is accepted by gastroenterologists in general, but there are lapses in the frequency of surveillance and in taking the requisite number of biopsies. Targeted biopsies may reduce this problem. Friedman *et al.*^[61] studied patient-related factors in colonoscopic surveillance and reported that only one-fourth of their patients underwent surveillance colonoscopy at an interval of less than three years; the factors related to non-adherence were logistics, health perceptions, stress regarding procedure, job or personal life, and procedural problems. The most frequent patient-related reason was difficulty with bowel preparation.

CONCLUSION

Should surveillance be continued in same way today or should we change it? It is clear that colonoscopic surveillance in the present form is neither an ideal nor practical approach. We feel that in the light of new data, the guidelines need to be re-examined. The surveillance should likely begin at six years after the onset of symptoms. It should consist of high-definition white light endoscopy with magnification chromoendoscopy and with targeted, rather than random, biopsies. The frequency of surveillance is not clear. In view of the recent comparison of American and British guidelines, further studies are necessary to decide frequency of surveillance. At present, British guidelines are useful, considering the fact that the risk of CRC is decreasing in UC. But there are ambiguities in both guidelines. As the technology evolves, it should be incorporated in surveillance (after considering cost-effectiveness): digital chromoendoscopy seems to come close to this. Other new technologies seem many years away.

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Diagnosis of inflammatory bowel disease: Potential role of molecular biometrics

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Abstract

Accurate diagnosis of predominantly colonic inflammatory bowel disease (IBD) is not possible in 30% of patients. For decades, scientists have worked to find a solution to improve diagnostic accuracy for IBD, encompassing Crohn's colitis and ulcerative colitis. Evaluating protein patterns in surgical pathology colectomy specimens of colonic mucosal and submucosal compartments, individually, has potential for diagnostic medicine by identifying integrally independent, phenotype-specific cellular and molecular characteristics. Mass spectrometry (MS) and imaging (I) MS are analytical technologies that directly measure molecular species in clinical specimens, contributing to the in-depth understanding of biological molecules. The biometric-system complexity and functional diversity is well suited to proteomic and diagnostic studies. The direct analysis of cells and tissues by Matrix-Assisted-Laser Desorption/Ionization

(MALDI) MS/IMS has relevant medical diagnostic potential. MALDI-MS/IMS detection generates molecular signatures obtained from specific cell types within tissue sections. Herein discussed is a perspective on the use of MALDI-MS/IMS and bioinformatics technologies for detection of molecular-biometric patterns and identification of differentiating proteins. I also discuss a perspective on the global challenge of transferring technologies to clinical laboratories dealing with IBD issues. The significance of serologic-immunometric advances is also discussed.

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Key words: Inflammatory bowel disease; Diagnosis; Advances and challenges; MALDI-MS/IMS; Molecular biometrics; Immunometrics

Core tip: Pouch surgery (the restorative proctocolectomy and ileal pouch-anal anastomosis for the curative surgical treatment of ulcerative colitis and familial adenomatous polyposis) replaces the colon and rectum after proctocolectomy with a pouch constructed from the distal small bowel (ileum) and sutured to the anal canal above the dentate/pectinate line preserving the anal sphincters. The operation restores gut continuity, defecation, deferral, and discrimination, if the diagnosis is correct, which is unpredictable in 30% of the colonic-inflammatory bowel disease-patients. Mass spectrometry and imaging mass spectrometry are groundbreaking, non-invasive analytical technologies with the ability to directly measure individual molecular species in complex clinical specimens. These technologies provide quantitative and qualitative analysis of cellular systems, and allow differentiation between disease and normal molecules from the same organ. These characteristics offer diagnostic and prognostic value for clinical medicine.

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INTRODUCTION

Inflammatory bowel disease

Colonic inflammatory bowel disease (IBD) comprises Crohn's colitis (CC) and ulcerative colitis (UC), a group of diseases of the gastrointestinal (GI) tract characterized by chronic relapsing and remitting inflammation^[1,2]. IBD affects as many as 1.6 million persons in the United States and 2.2 million in Europe. The incidence is increasing worldwide^[1-3]. In spite of advances in IBD-therapy, IBD hospitalizations and surgery rates in the United States have increased significantly since 1990^[6]. IBD is one of the five most prevalent GI disease burdens in the United States, with annual overall health care costs of more than \$1.7 billion^[7,8]. One to two of every 1000 people in developed countries are affected with IBD^[9], and global rates seem to be increasing^[1,10-12], attributable to the rapid modernization and Westernization of the population^[1]. These chronic diseases result in significant morbidity and mortality, compromising quality of life and life expectancies. While there is no drug for cure for these diseases, the last three decades have seen major advances in the molecular understanding intestinal immune responses and how they relate to IBD. This, in turn, has led to the development and refinement of several new treatments. Most significant has been the development of restorative proctocolectomy (RPC) with ileal pouch-anal anastomosis (IPAA). The pelvic pouch surgery allows for the removal of the entire colon while maintaining transanal fecal continence without a permanent diverting loop ileostomy. The success of RPC (judged by the entire removal of a diseased colon while preserving gastrointestinal continuity, bowel evacuation, continence and fertility) restores physiological function and greatly improves patient health quality of life. Successful RPC also frees the healthcare system from the immense burden of current lifelong, non-curative treatments. These outcomes are dependent on a correct diagnosis and meticulous surgical techniques available at well-established IBD centers^[13-15].

The etiology of IBD poorly understood. The general consensus holds that IBD is an automatic dysfunction triangle of antigen and antibody reaction against mucosal response to commensal bacteria. The fundamental question is why the immune system responds aggressively to harmless, ever-present bacteria, releasing complex mixes of cytokines, chemokines and other substances that cause inflammation. One possible explanation is that the gut immune system is compromised because of defects in the barrier function of the gut luminal epithelium^[16]. Although the etiology of IBD is at present not delineated, histopathologic and clinical assessments demonstrate that CD and UC, the two major classifications of IBD,

are indeed distinct entities and have different causes and discrete mechanisms of tissue damage and treatment^[16-21]. UC results in inflammation and ulcerations in the mucosal and to a lesser degree submucosal linings of the colon and rectum. CD differs in that it may result in inflammation deeper within the intestinal wall (transmucosal) and can occur in any parts of the digestive system (including the mouth, esophagus, stomach, duodenum, small intestine, colon and rectum). Further, Crohn's may also involve other organs outside the GI system through fistulization^[22,23]. Crohn's is diagnosed in at least four patients per 100000 in the United States, and the incidence and prevalence is rising worldwide^[1,10-12].

Diagnosis challenges in IBD

The current standard of care for IBD treatment is based on steroids and immunosuppressant agents, including glucocorticoids, aminosaliclates, cyclosporine, methotrexate and biologic agents such as anti-TNF- α and IL1- β . The correct IBD diagnosis is crucial for providing correct, evidence-based treatment, since treatment response and complications differ significantly among UC and CC patients^[24]. The absence of specific phenotypes indicating the particular disease condition challenges pathologist interpretation and categorization of tissue morphology, subsequently leading to difficulties in diagnosis and consistent standard of care^[25]. However, despite advances in our understanding of the genetic^[16,26], immunologic^[26,27], and environmental^[1,24,28] influences that may trigger complex IBD pathologies, to date there is no single indicator sensitive enough to accurately and consistently delineate CC and UC. The available data indicate that genetic factors determine an individual's susceptibility to developing IBD, and environmental factors elicit cellular responses that drive disease progression. Histological evaluation and interpretation of tissue provides insights that directly impact care^[25]. Pathologists rely mainly on microscopic visual inspection and interpretation of stained and/or dyed tissue sections to identify the disease state of a patient sample^[29,30]. Inherently, these procedures possess a significant degree of subjectivity^[31] and are fraught with problems^[31,32]. Rigorous training in pathology subspecialties has attempted to improve the standard of care and avoid unnecessary mistakes^[33]. Despite these extremely thorough standards, inevitable situations arise in which objectivity cannot be guaranteed and where significant disagreement occurs between specialists^[34]. This challenge is common for IBD patient populations^[13,15,35,36]. To date, there is no single, absolute diagnostic test^[37,38]. A diagnosis should neither be based on nor excluded by any one variable or result^[39]. The consensus statement on the diagnosis, management and surveillance of both CC^[40] and UC^[41] recommend that "multiple" tissue biopsies from at list five sites around the colon and rectum should be collected for support of a reliable diagnosis. Of these six sites a minimum of two samples from each should be sampled^[40,41]. Although the procedure is reliable, it is invasive and uncomfortable to the patients.

Table 1 Microscopic features used for the diagnosis of Crohn's colitis

Colon	
Architecture	
Crypt architectural irregularity	Focal
	Diffuse
Reduces crypt numbers/mucosal atrophy	
Irregular surface	
Chronic inflammation	
Distribution I	Focal increased in intensity
	Patchy increase
Distribution II	Diffuse increase
	Superficial
Granulomas	Transmucosal
Mucin granulomas	Basal plasma cells
Polymorph inflammation	
Lamina propria	Focal
Crypt epithelial polymorphs	Diffuse
Polymorph exudates	
Epithelial changes	
Erosion/ulceration	
Mucin	Depletion
	Preservation
Paneth cells distal to hepatic flexure	
Epithelial-associated changes	
Increased intraepithelial lymphocytes > 15	
Terminal ileum/Ileocecal /Cecum	
Architecture	Villus irregularity
	Crypt architecture
Epithelial changes	Irregularity
	Pseudopyloric gland
	Metaplasia

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Inaccurate diagnosis in IBD and consequences

When IBD predominantly involves the colon, differentiation between CC from UC is often challenging. Inaccurate diagnoses are estimated to occur in 30% of IBD patients^[42,43]. In most cases the diagnostic uncertainty arises from the overlap of clinical and histologic features, making CC appear like UC^[44]. This scenario is particularly relevant to young children, a population in which IBD consists of up to 80%. The differentiation between UC and CC relies on a compilation of clinical, radiologic, endoscopic, and histopathologic interpretations^[40], a compilation that is not always accurate. An estimated 15% of IBD patients are indistinguishable and are labeled as “indefinite colitis” (IC)^[45-47]. In addition, another 15% of the colonic IBD cases that undergo pouch surgery resulting from a definitive UC diagnosis (based on the pathologist's initial designation of endoscopic biopsies and colectomy specimen) will have their original UC diagnosis changed to CC based on the postoperative follow-up when clinical and histopathology changes indicate development of CC in the ileal pouch^[15,35,36,48,49]. One-half of these patients will require pouch excision or diversion^[49].

Because of the unpredictable nature of IBD, side effects of medications, and potential complications, some of which may end in sudden incapacitation, IBD is be-

coming a global health concern. Distinguishing between CC and UC is critical to therapy. The clinical experience suggests that identifying patients with CC and positive outcomes after pouch surgery is arduous. Thus, RPC should be contraindicated for CC patients, whereas IPAA is standard acceptable care for patients with UC and IC who are predicted likely to develop UC. Inevitably, pouch complications are significantly higher in patients with CC ($\pm 64\%$) and IC ($\pm 43\%$) *vs* patients having UC ($\pm 22\%$) ($P < 0.05$)^[46,47,49]. This diagnostic dilemma and the potential morbidity from a wrong diagnosis and unnecessary and/or inappropriate surgical interventions underscore the importance of research strategy focused at improving diagnosis of the colitides using molecular biometrics^[42,50-52].

Clinico-histopathologic findings in Crohn's colitis

Crohn's colitis is recognized to encompass a heterogeneous group of disorders^[38]. Usually CC is segmental with deep inflammation where the disease activity is transmural, with lymphoid composite extending to the sub-serosa. The Montreal classification^[53] and the Paris pediatric modification^[54] have brought consistency to definitions of subtypes of CC and of colitides. It is noteworthy that both the Montreal and Paris classifications rely on the location of gross disease, *i.e.*, visible lesions with more than a few aphthous ulcers. Patterns of macroscopic involvement, rather than microscopic, have been useful traditionally in predicting clinical course, as exemplified by the tendency of small bowel disease, particularly, to stricture over time. Despite the fact that microscopic involvement does not define subtypes of CC, the role of histology in the diagnosis of CC does differ according to the anatomic location of macroscopic disease^[38].

Histologic features useful for the diagnosis of CC have been reviewed by Griffiths^[38], (Table 1) but, according to Van Assche *et al*^[40] presented at The second European evidence-based Consensus on the diagnosis and management of Crohn's colitis, there are no data available as to how many of these features must be present to allow a firm diagnosis^[40]. Focal (discontinuous) chronic (lymphocytes and plasma cells) inflammation and patchy chronic inflammation, focal crypt irregularity (discontinuous crypt distortion) and granulomas (not related to crypt injury) are the generally accepted microscopic features which allow a diagnosis of CC^[40]. Within one histologic section, inflammation may be immediately adjacent to an uninfamed microscopic “skip area”. Mucosal changes may resemble ulcerative or infectious colitis with infiltration of the crypts by polymorphonuclear leukocytes (cryptitis or crypt abscesses), and distortion of crypt architecture. Granulomas (collections of monocytes/macrophages) in the lamina propria (not associated with crypt injury) are a corroborating feature of suspected Crohn's after exclusion of identifiable infectious etiology, but reported prevalence in mucosal biopsies at time of first diagnosis varies. The likelihood of finding granuloma is a function of the number of specimens taken, the number of sections examined,

Table 2 Classic microscopic features in untreated ulcerative colitis (comparable Crohn's colitis, hard criteria)

Feature	Ulcerative colitis	Crohn's colitis
Diffuse	Continuous disease	Segmental disease
Rectal	Involvement	Variable rectal involvement
Disease	Worse distally	Variable disease severity
Fissures	No	Fissures, sinus, fistula
Transmural aggregates	No	Transmural lymphoid aggregates
Ileal involvement	No, exception during backwash ileitis	Ileal involvement
		Upper gastrointestinal involvement
Granulomas	No	Granulomas

and the definition of a granuloma. Granulomas occur more commonly in the submucosa than the mucosa^[55]. Hence, they are observed in 60% of surgical specimens but relevant to the question of histology for diagnosis, in only 20%-40% of mucosal biopsies^[55]. Moreover, according to Griffiths^[38] data indicating clinical significance or prognostic value of presence or absence of granulomata are lacking.

Clinico-histopathologic findings in ulcerative colitis

The classic microscopic features in untreated UC (and CC hard criteria) used for diagnosis, as outlined by Odze^[56], and are depicted in Table 2. Clinically, the hallmark of UC is hematochezia^[57,58]. Additional clinical presentations include rectal tenesmus and incontinence, abdominal pain, severe inflammation of the rectum (proctitis), leukocytosis, hospitalization for total parenteral nutrition and/or intravenous fluids correction, among others. Blood transfusion and corticosteroids are recommended when considering surgery (RPC and IPAA)^[58]. As mentioned earlier, in UC, inflammation is typically confined to the mucosal layer and to the lesser degree to the submucosa. Children with UC often have evidence of chronicity, rectal frugality, and little or no architectural warping. In otherwise usual cases of UC, these conditions may lead to a confusion with CC^[59-61].

Current advances in biomarker discovery to delineate the colitides

To date, there has been significant interest in attempting to identify molecular biomarkers that can accurately delineate CC and UC phenotypes. These studies have been minimally successful at identifying such biomarkers. In serum these include: placenta growth factor-1 (PLGF-1), IL-7, TGFβ1, and IL-12P40^[62-67]. In biopsies obtained from the mucosa, they are Rho GD1α, desmoglein, pleckstrin, VDAC (voltage-dependent anion channel), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), and C10orf76^[68,69]. In stool they are calprotectin, PMN-elastase, lactoferrin, and S100A12^[65,70-74]. Clearly these biomarkers represent an advance in the field of colitides research and have been used for clinical prognostic trials but have not been shown to delineate UC from a CC phenotype^[62,64,73,74]. Thus far, the above mentioned features reflect colitides intestinal inflammation and do not discriminate UC from the CC

phenotype^[65].

Histology-directed proteomic advances

Histology-directed MALDI MS is the first attempt ever used to analyze and compare mined proteins of the colonic mucosal and submucosal tissue layers individually, in order to differentiate between UC and CC^[42,50]. The normal *topography* of the *colon* and the layers used in mining and extraction of analytical extracts are illustrated in Figure 1. The basic steps of the methodology of histology-directed mass spectral protein profiling are outlined in Figure 2. Specialized MALDI MS offers directly the possibility of direct proteomic assessment of the tissue itself. The histologic layers of colectomy samples from patients with histologically and clinically confirmed UC and CC, with no ambiguity, are analyzed individually using MALDI MS for proteomic profiling. The results have successfully identified highly significant MALDI MS mass-to-charge ratio (m/z) signals in colonic tissue layers that appear to be phenotype-specific and are likely to help distinguish UC and CC^[42,50]. Pre-sequencing and identification proteomic pattern peaks from colonic mucosal or/and submucosal tissue section are depicted in Figure 3^[50]. These signatures do not correlate to tissue of origin and thus represent disease-specific markers. Some of these are found in colonic mucosa, from which endoscopic biopsies could be subjected to proteomic analysis. Other signatures come from the submucosa and could be used for proteomic studies of serum. Other protein-signatures were found in both tissue layers. Identifying proteomic patterns characteristic of one specific colitis phenotype will significantly improve our understanding of the mechanistic events associated with IBD.

It is unlikely that a single protein or small cluster of proteins will have the necessary: (1) specificity; (2) sensitivity; (3) discrimination; and (4) predictive capacity, to differentiate the heterogeneity of IBD^[69]. However, if it were possible, it would require a technology that can accommodate sampling large patient cohorts, while accounting for patient variability. MS is an important profiling and identification tool for such studies^[75]. As necessary as the tool is, subsequent analysis and validation methods will determine the actual success of a detection system intended for non-invasive screening and evaluating treatment efficacy. The overall goal of delineating IBD by proteomics is to illuminate the pathobiology underlying the colitides. More

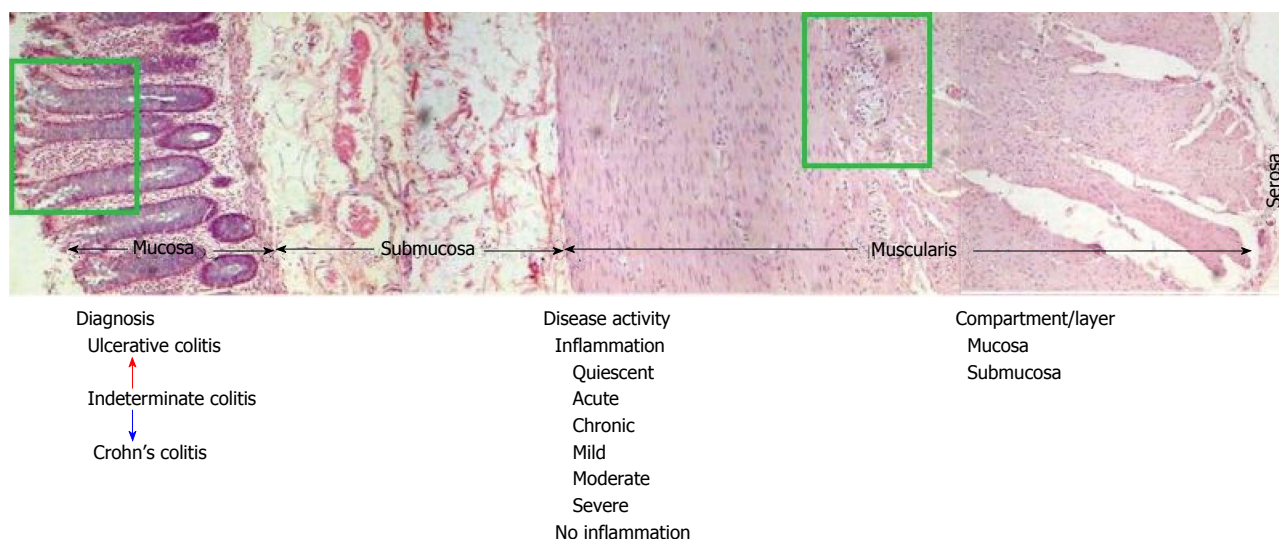


Figure 1 Human colon cross section depicts layers for mining proteomic patterns that delineates untreated ulcerative and Crohn's colitis phenotype. The colon is comprised of four distinct layers: (1) the mucosa; (2) the submucosa; (3) the muscularis (two thick bands of muscle); and (4) the serosa. Comparable proteomic patterns that are mined from these layers are analyzed, based on the diagnosis [untreated ulcerative and Crohn's colitis, (with no ambiguity)], disease activity and tissue layer.

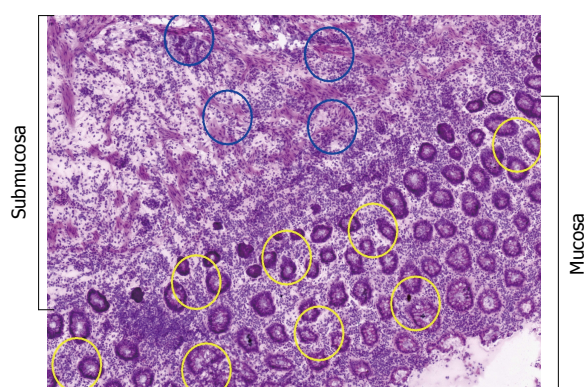


Figure 2 Histology-directed tissue layer profiling for matrix-assisted-laser desorption/ionization mass spectrometry. Digital photomicrographs acquired from histology and matrix-assisted-laser desorption/ionization sections were used to identify and designate sites of interest for profiling. Comparisons were performed in both the training and independent test set samples between inflamed mucosa Crohn's colitis (CC) vs ulcerative colitis (UC) and inflamed submucosa CC vs UC. Tissue section showing marked areas of pathological interest. Rings demonstrate matrix spots in mucosal and sub-mucosal layers (unpublished figure).

specifically, it is to identify patterns differentiating the colonic IBDs that exhibit overlapping clinical and histologic signs, but require different approaches of care. The anticipation is that this approach will eventually provide molecular biometrics of interest that can tell UC from CC through endoscopic biopsies and eventually create a serum biomarker tool assay for the identified peptide, if the protein(s) is (are) secretory and transposable. Better understandings of the bio-pathophysiologic mechanisms may allow new therapeutic and preventive avenues for maintenance or remission in IBD.

Matrix-assisted laser desorption/ionization MS

Specialized matrix-assisted laser desorption/ionization

(MALDI) MS offers the possibility of direct proteomic assessment of the tissue itself^[76]. The molecular specificity and sensitivity of MS can image and map biomolecules present in tissue sections. Applying complementary techniques of immunochemistry and fluorescence microscopy to MALDI MS data can improve the analysis of spatial arrangements of molecules within biological tissues. Accordingly, MALDI technology has become a popular in biology research. It combines two technologies, the MALDI “soft” ionization source and the TOF (Time of Flight) mass analyzer. The former volatilizes and ionizes molecules using a laser, a target, and an organic compound called a matrix, while the latter technology measures an ion's mass-to-charge ratio (m/z) by measuring the time it takes to reach a detector. MALDI TOF mass spectrometers come in two basic types: MALDI TOF MS and MALDI TOF/TOF MS. The latter enables tandem mass spectrometry (MS/MS) studies^[69]. Thus a combination of markers may improve the chances of achieving IBD proteomics goals.

MS in combination with laser capture microdissection is another important profiling and identification tool for such studies. It allows direct tissue analysis of biomolecules and large organic molecules which are often too fragile for conventional ionization methods. These techniques may significantly enhance diagnostic accuracy and provide the basis for future bio-physiologic elucidations in IBD.

MALDI IMS

MALDI IMS stands out as a tool for imaging metabolites in the biological and medical fields, and as a new tool for pathology in the molecular age^[77]. There are several advantages in IMS technology. First, IMS does not require labeling or specific probes. Second, it is a non-targeted imaging method, meaning unexpected metabolites can

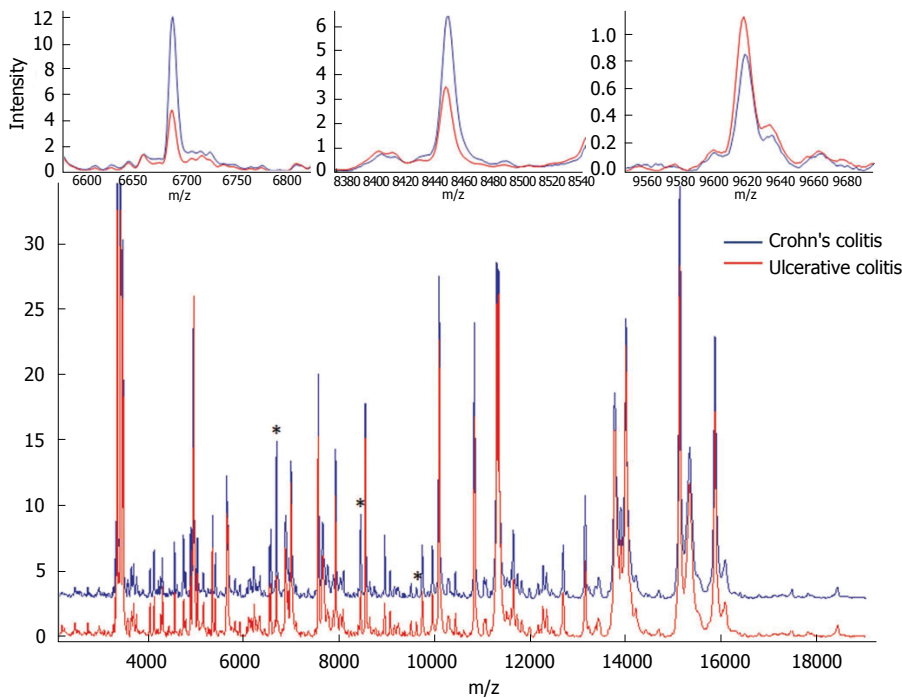


Figure 3 Show averaged mass spectrum proteomic pattern spectra from Crohn's colitis (blue) and ulcerative colitis (red). Differential distribution of three selected proteomic pattern peaks (m/z) obtained from colonic mucosal and/or submucosal tissue sections that were part of the Support Vector Machine model. They are denoted by "a" symbol in the full spectra. Reproduced with permission from the publisher: Seeley *et al*^[60].

easily be imaged. Finally, several kinds of metabolites can be imaged simultaneously. The technique effectively provides a better visualization of the underlying mechanisms of biological processes of endogenous, small metabolites^[78,79] and large proteins^[80,81] in cells and tissues^[82,83]. It can determine the distribution of hundreds of unknown compounds in a single measurement^[79,84-86]. Further, IMS is capable of three-dimensional molecular images which can be combined with established imaging techniques like magnetic resonance imaging^[87,88].

Due to the fact that the enormous molecular diversity of metabolite species is unknown, IMS technology is seemingly appropriate for localizing metabolites, whether they are from the molecule of interest or not^[78,89,90]. The emerging technique of MALDI IMS has the capability to distinguish between parent and metabolites while maintaining spatial distribution in various tissues^[91,92]. In spite of the promising advances of MALDI IMS for visualizing tiny metabolites, substantial concerns remain regarding its spatial resolution. The primary limitation results from the size/volume of the organic matrix crystal and analyte migration during the matrix application. There is also a lack of efficient computational techniques for constructing, processing, and visualizing large and complex 3D data which prevents experimenters from tapping its full potential^[93]. In attempting to solve these important issues, researchers have devised another sophisticated method: a nanoparticle-assisted laser desorption/ionization (nano-PALDI)-based IMS, in which the matrix crystallization process is eliminated^[94,95]. The use of novel nano-PALDI has enabled scientists to image compounds with spatial resolution at the cellular level (15

μmol/L; approximating the diameter of a laser spot)^[96].

Serologic test advances

To date, a lack of validated information prevents recommending the use of serologic assays to screen general population patients for undiagnosed gastrointestinal symptoms in IBD-settings. As has been made clear, no unique biomarkers yet exist for the delineation between CC and UC. Serologic tests, antineutrophil cytoplasmic antibodies (ANCA) and anti-microbial antibodies are inadequately sensitive and specific to contribute much to the diagnosis of CC or to its differentiation from UC.

ANCA are immunoglobulin G (IgG) antibodies directed against cytoplasmic components of neutrophils^[97]. The association with colitides of a subset of ANCA with a perinuclear staining pattern on immunofluorescence studies [perinuclear antineutrophil cytoplasmic autoantibodies (pANCA)] was first recognized for UC, where it was detected in 60%-70% of patients^[97]. The specificity of perinuclear staining for colitides can be validated and confirmed by its disappearance after deoxyribonuclease (DNase) digestion of neutrophils. pANCA is considered a marker of the immunologic disturbance that underlies the development of chronic colonic inflammation, and should not be positive in acute self-limited, presumably infectious colitis.

Anti-*Saccharomyces cerevisiae* antibodies (ASCAs), the first anti-microbial antibodies to be described in CC, are IgG and IgA antibodies that recognize mannose sequences in the cell wall of *S. cerevisiae* strain Su1. ASCA is detected in 50%-70% of CC patients overall, 10%-15% of UC patients and in 5%-10% of controls with other

gastrointestinal disorders^[97]. Newer anti-microbial antibodies (Abs), which include Abs against *Pseudomonas fluorescens*-associated sequence (anti-I2), anti-outer membrane protein C of *Escherichia coli* (anti-OmpC), anti-outer membrane protein of *Bacteroides caccae* (anti-OmpW), and anti-flagellin Abs (anti-CBir1), may result false positive and be detected in patients who otherwise have negative serology, but are nonspecific and can be detected in patients with other diseases^[98,99].

Differentiation of CC from UC is clinically problematic because inflammation is only confined to the colon. pANCA is positive in up to 35% of patients with CC; ASCA is less often detected in patients with CC. Hence, the utility of combined ANCA/ASCA testing is less in the setting where it is needed most. In the one published study clearly reporting sensitivity, specificity, and predictive values of combined serologic testing, the sensitivity of ASCA+pANCA-serology for CC *vs* UC was only 32%^[97]. In a long-term follow-up of patients with IC, Joossens *et al*^[100] observed 26 patients who were ASCA+/pANCA- at baseline. Eight were later diagnosed with CC and 2 with UC, while the other 16 patients remained IC. The ASCA-/pANCA+ profile was even less helpful for definitive diagnosis^[100].

When using upper GI biopsies, the differentiation between UC and CC is relatively straightforward in most of patients. In appropriate clinical settings, granulomatous inflammation in GI biopsies validates CC. In pediatric CC, granulomas may only be found in biopsies from the upper GI. Without routine upper endoscopy, these cases will be missed. If granulomas are not found, a diagnosis of CC or UC can be derived from endoscopic findings with histology combined with clinical and imaging determinations^[101]. Determining cases of IBD as CC, UC, or IC is largely a matter of nomenclature. Supporting a determination with evidence from endoscopies, magnetic resonance enterography, or other techniques, improves clinical labelling of the condition. The colitides are a continuum between CC and UC, with a variety of inflammations between. Teasing out overlapping genetic profiles for UC and CC will be critical to applying correct treatment more accurately than using current nomenclature categories based on a current standard of histology^[100]. Application and refinement of the above technologies and techniques will improve the possibility of approaching patients with individualized options reducing ineffective or unnecessary surgery. Usage of molecular biometrics to differentiate diseases of the same organ^[38,102,103] is becoming ground breaking in improving diagnostic challenges in colonic IBD settings^[42,50,104]. IBD has no permanent drug cure and results in significant morbidity and mortality^[9,104,105]. UC is absolute colonic disease while CC can involve any part of the GI system from the mouth to the anus, which may transmurally involve partial to a full-thickness of the intestinal wall^[43] and other organs through fistulization^[106-108]. These diseases share several clinical biometric signatures but have different causes, mechanisms of tissue damage, and treatment options^[16,109]. Therefore, accurate diagnosis is paramount

for provision of correct pharmacologic therapy^[110,111] and surgical care^[112-114].

CONCLUSION

The term “colitides” characterizes colonic IBD and comprises ulcerative colitis and Crohn’s colitis (UC and CC). The etiopathogenesis of UC and CC remains enigmatic. Diagnostic accuracy for distinguishing these two pathologies is still a significant problem in GI medicine and is hindered by a growing overlap of histopathological interpretation. Despite all efforts, many patients continue to remain undetermined as UC or CC, and are said to have indeterminate colitis. Differentiations of UC and CC are concluded from imprecise clinical, histopathologic, and other examinations. This results in speculative colitis staging and severity which cannot be conclusively differentiated in up to 30% of patients with IBD. CC and UC diagnostic features often overlap^[115] even after a thorough histological assessment, the current gold-standard for distinguishing type of inflammation (for CC: lack of non-specific inflammation not confined beyond mucosa and diffused or focal granulomatous *etc.* For UC: inflammation limited to the mucosa, diffuse infiltration of acute and chronic inflammatory cells in the mucosa, continuous damage from the rectum to proximal colon, *etc.*).

Treatment options for UC and CC differ significantly. Thus appropriate individualized prognosis and treatment requires accurate diagnosis. An estimated 90% of patients with IC undergo pouch surgery (RPC and IPAA) for fulminant colitis^[36,48,49,115,116] contrasting with 30% of patients in whom UC or CC was a correct diagnosis. Additionally, failure to recognize specific indicators of CC (*e.g.*, granulomas and transmural inflammation) often leads to mistakes in pathological interpretation^[24,36]. This results in a reciprocal misdiagnosis rate of 15% (CC as UC: UC as CC). Adding = the 15% of cases labeled as IC accounts for nearly a third of the all IBD patients. Those undergoing surgery for a presumably confirmed diagnosis of UC subsequently are diagnosed postoperatively with recurrent CC in the ileal pouch^[36]. This is critical because functional failure and higher complication rates are estimated at up to 60%^[35,117-123] and often require excision of the pouch with a permanent end ileostomy^[35,121-124]. At this stage, patient health quality of life is significantly jeopardized for life.

There has been wide ranging interest in attempting to identify molecular biomarkers that can consistently delineate these diseases. These studies have been minimally successful at identifying quiescent or active IBD in serum^[62-67], in mucosal biopsies^[68,69], and in fecal matter^[65,70-74]. Clearly these features represent an intriguing advance in the science of IBD for clinical disease prognostic purposes. However, these markers have not been shown to distinguish UC from CC phenotype^[62,64,73,74]. A serology panel including ANCA, pANCA, anti-saccharomyces cerevisiae IgG and IgA antibodies (ASCA), calgranulin (S100A12), anti-OmpC antibodies, fecal lactoferrin, calprotectin, and polymorphonuclear neutrophil

elastase (PMN-e)^[65] is marketed as a promising approach to monitor disease activity and prognosis and may prove to be beneficial in the management of IBD. The specificity, sensitivity and diagnostic accuracy of these parameters with reference to clinical disease indices and/or endoscopically measured inflammation in IBD setting remain unclear. What we have learned to date is that: (1) Although not yet commercially available as tests, patients with CC are more likely than healthy control and/or IBD patients to be positive for a range of biomarkers such as S100A12 (calgranulin), ASCA, OmpC, CBir1, pseudomonas fluorescens protein, and pANCA^[125,126]. Significant increases of these proteins are noted during active intestinal inflammation. The greater the number of positive serologies and the higher the titer, the more aggressive the course. These biomarkers are also seen in an active UC^[127]; (2) A combination of these biomarkers and a disease-specific activity index could promote the diagnostic accuracy in clinical medicine with reference to endoscopic inflammation but at present none are superior in the ability to reflect endoscopic inflammation^[70]; (3) These molecular biometrics significantly assist in predicting relapses in patients with confirmed IBD (active or quiescent)^[2-5,17,21,128] but are not discriminatory between UC/CC; (4) Patients who are pANCA+ and ASCA- are more likely to have UC than CC, while in pANCA- and ASCA+ patients the reverse may be true^[67]. However, these biomarkers have not demonstrated clinical utility as predictors or monitoring tools of IBD activity^[67].

At the present time there is insufficient biometric information to recommend use of serologic assays in screening for IBD in patients from the general population who have undiagnosed gastrointestinal symptoms. Further, no efficacy for the delineation of CC and UC clearly exist.

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