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REVIEW

Irritable bowel syndrome in children: Current knowledge, challenges and opportunities

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Abstract

Irritable bowel syndrome (IBS) is a common and troublesome disorder in children with an increasing prevalence noted during the past two decades. It has a significant effect on the lives of affected children and their families and poses a significant burden on healthcare systems. Standard symptom-based criteria for diagnosis of pediatric IBS have changed several times during the past two decades and there are some differences in interpreting symptoms between different cultures. This has posed a problem when using them to diagnose IBS in clinical practice. A number of potential patho-physiological mechanisms have been described, but so far the exact underlying etiology of IBS is unclear. A few potential therapeutic modalities have been tested in children and only a small number of them have shown some benefit. In addition, most of the described patho-physiological mechanisms and treatment options are based on adult studies. These have surfaced as challenges when dealing with pediatric IBS and they need to be overcome for effective management of children with IBS. Recently suggested top-down and bottom-up models help integrating reported patho-physiological mechanisms and will provide an opportunity for better understanding of the diseases process. Treatment trials targeting single treatment modalities are unlikely to have clinically meaningful therapeutic effects on IBS with multiple integrating patho-physiologies. Trials focusing on multiple combined pharmacological and non-pharmacological therapies are likely to yield more benefit. In addition to treatment, in the future, attention should be paid for possible prevention strategies for IBS.

Key words: Abdominal pain; Functional gastrointestinal disorder; Irritable bowel syndrome; Management; Microbiota; Patho-physiology; Post-infectious

Core tip: Even though irritable bowel syndrome (IBS) is a common worldwide pediatric problem, little is known of its exact patho-physiology and management. Therefore, a large number of children are suffering from its intestinal and extra-intestinal symptoms. Novel research using new advanced technologies based on proposed top-down and bottom-up models of patho-physiology and treatment trials focusing on multiple combined interventions are likely to be more beneficial in understating and treating pediatric IBS. In addition, the time has come to explore possible prevention strategies for this problem.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common pediatric functional gastrointestinal disorder (FGID)[1-10] with severe disabling upper and lower gastrointestinal symptoms[11] and extra-intestinal symptoms[1,11]. It has a significant impact on daily activities[2,11,12], education[11-13] and health related quality of life[9,10,13,14] of affected children. The healthcare consultation rate is high in affected children[1,9,15] and it leads to a significant annual healthcare cost[15-17].

Unlike many other gastrointestinal disorders, IBS and other FGIDs have major challenges in terms of diagnosis, underlying patho-physiology and management. In the absence of detectable biomarkers, IBS is a purely symptom based diagnosis[18]. However, the accepted diagnostic criteria for IBS have changed several times in the past[18-20] and are quite likely to change in the future. Keeping up with these changes is a major test for both clinicians and researchers dealing with IBS and in the past numerous disagreements have been reported even among medical professionals in terms of interpreting them[21,22]. Numerous mechanisms have been suggested as possible underlying causes for symptoms of IBS[23]. During the past decade the number of research on pediatric neuro-gastroenterology have increased at a global level and more and more studies have been conducted using non-invasive, sophisticated, cutting edge technologies to understand motility of the digestive tract, intestinal microbiota, underlying genetic and epigenetic mechanisms, gastrointestinal signaling molecules and different areas of the brain in relation to stimuli from the gut[23]. However, up to now these findings have failed to give a clear holistic picture on the underlying patho-physiology of IBS. Without a clear and proven understanding of the patho-physiology, it is not easy to design and conduct effective clinical trials for IBS. In addition, the therapeutic options of managing IBS is not all that well researched in children[24]. With all the research and clinical interest on non-communicable diseases including IBS around the globe, and emerging novel investigation techniques, there are more opportunities to understand the likely patho-physiology of childhood IBS, develop more therapeutic modalities to support children and moreover, to develop potential preventive methods.

In this review, we have attempted to highlight the main challenges concerning the diagnosis, patho-physiology and management of childhood IBS while summarizing the current knowledge on epidemiology, risk factors, patho-physiology, diagnosis and management. Furthermore, we have also outlined a road map for possible directions for future research which would of benefit to children with IBS.

DEFINITION OF IBS

IBS is a symptom based diagnosis. Therefore, defining the exact symptom based diagnostic criteria for childhood IBS has always been a major challenge.

Rome IV criteria for IBS

At the beginning, all abdominal pain disorders in children were classified into one group labelled as recurrent abdominal pain by John Apley. He defined it as at least three episodes of abdominal pain, severe enough to affect their activities over a period longer than three months[25]. This definition was accepted by both clinicians and researchers all over the world for almost five decades. The formation of the Rome Foundation paved the way for developing a new diagnostic classification system for functional abdominal pain disorders. Introduction of Rome Criteria in 1989 for chronic abdominal pain in adults provided the opportunity to use the same criteria for the pediatric age group and it was demonstrated that a large proportion of children with chronic abdominal pain also fulfilled the adult criteria for IBS[26]. Based on these findings, Rome Ⅱ criteria released in 1999, and subsequently the Rome Ⅲ criteria released in 2006, included symptom-based diagnostic criteria for IBS in children[19,20]. Pediatric Rome criteria gained popularity immediately after they were released and have been adopted in subsequent research conducted in IBS[27-30]. However, both Rome Ⅱ and Rome Ⅲ criteria had their own deficiencies which were often directed to the difficulties in reporting and interpreting the symptoms between different cultures, populations, communities and social classes. Schurman *et al*[28], comparing the child report, parental report and the physician diagnosis, showed a diagnostic disagreement between the 3 groups when they used the Rome Ⅱ criteria. Most of the time the reasons for the discrepancies in the diagnoses were due to disagreement on symptoms related to defecation. The diagnostic agreement (both Rome Ⅱ and Rome Ⅲ)

Table 1 Rome IV criteria for irritable bowel syndrome in children and subtypes

Diagnostic criteria for irritable bowel syndrome (IBS)[18]
Must include all of the following
Abdominal pain at least 4 d per month associated with one or more of the following:
Related to defecation
A change in frequency of stool
A change in form (appearance) of stool
In children with constipation, the pain does not resolve with resolution of the constipation (children in whom the pain resolves have functional constipation, not irritable bowel syndrome)
After appropriate evaluation, the symptoms cannot be fully explained by another medical condition
Above criteria needs to be fulfilled for at least 2 mo before diagnosis.
Diagnostic criteria for IBS subtypes[31]
IBS with predominant constipation
More than one-fourth (25%) of bowel movements with Bristol stool form types 1 or 2 and less than one fourth (25%) lf bowel movements with Bristol stool form types 6 or 7
IBS with predominant diarrhea
More than one-fourth (25%) of bowel movements with Bristol stool form types 6 or 7 and less than one fourth (25%) lf bowel movements with Bristol stool form types 1 or 2
IBS with mixed bowel habits
More than one-fourth (25%) of bowel movements with Bristol stool form types 1 or 2 and more than one fourth (25%) lf bowel movements with Bristol stool form types 6 or 7
IBS unclassified
Patients who meet diagnostic criteria for IBS but whose bowel habits cannot be accurately categorized into 1 of the 3 groups above

was also poor when pediatric gastroenterologists were compared to gastroenterology fellows[21,22].

The deficiencies mentioned above, the expansion of knowledge and better understanding of childhood FGIDs paved the way for development of Rome Ⅳ criteria[18], released in 2016, after summarizing a decade of research on FGIDs[18]. In that venture, the label of abdominal pain related functional gastrointestinal disorders was replaced by the term functional abdominal pain disorders (FAPDs). In addition, the frequency of pain symptoms was differently worded from once a week for at least 2 mo in Rome Ⅲ to at least 4 d per month for at least 2 mo[18]. However, the 4 main FAPDs; IBS, functional dyspepsia (FD), abdominal migraine (AM) and functional abdominal pain not specified (FAP-NS) remain the same. The new Rome Ⅳ criteria for IBS are summarized in Table 1. Exact validity of these Rome Ⅳ criteria need to be studied in the future.

Sub-types of IBS

The sub-categories of IBS in children [IBS with predominant diarrhea (IBS-D), IBS with predominant constipation (IBS-C), IBS with mixed bowel habits (IBS-M) and IBS unclassified (IBS-U)] are included in Rome Ⅳ for the first time, in parallel with adult criteria (Table 1)[31]. Subcategories are very important in the management of affected children.

EPIDEMIOLOGY OF IBS

IBS is often reported as one of the commonest FGIDs in children. A recent meta-analysis conducted on epidemiologic studies on abdominal pain from 1957 to 2014, noted IBS in 8.8%, FD and FAP in 4.5% and 3.5% respectively. Another systematic review and meta- analysis on IBS in Asian children showed a higher prevalence of 12.4%[32]. Several studies from Greece, Nigeria, South America and Sri Lanka have recognized IBS as the most prevalent FGID among children and adolescents (2.9%, 9.9%, 3.8%-6.4% and 3.6%-7% respectively)[6-9,33-36]. Many other studies have also reported a high prevalence of IBS in China (13.25%)[1], Nigeria (16%)[2] and Turkey (22.6%)[3]. In contrast to this, studies from United States have shown lower prevalence rates of IBS (2.8% and 5.1%)[37,38]. A recent study from the Mediterranean region also reported IBS in 4% of children and adolescents[39]. However, it is not clear whether the differences in reported prevalence are true differences or are due to the differences in interpreting Rome criteria between different cultures in terms of pain characteristics and bowel habits.

Different subgroups of IBS have different bowel symptoms and because of that, the exact approach to the management differs between subgroups. Therefore, recognizing sub-types of IBS is of utmost importance in effective management of children with this conditions. Even though IBS sub-groups were only recently recognized in Rome Ⅳ criteria, in the past some researchers have used adult criteria to differentiate between sub-groups of IBS in children. A prospective, hospital based study from Italy reported IBS-C as the most prevalent IBS sub-type (45%) followed by IBS with mixed bowel habit and IBS unspecified (29%), and IBS-D (26%)[12]. Some other studies have reported IBS-C as the most common IBS sub-type too[3]. However, two more recent epidemiological from Greece and Nigeria reported higher prevalence of IBS-M (47.9% and 53.6% respectively). In these two studies IBS-D and IBS-C were noted in 16.7%-19% and 27.4%-6.3% respectively[2,33]. In contrast, an Asian study noted an almost similar prevalence of IBS-C, IBS-D and IBS-M (29%-30%)[11]. The changing nature of IBS subtypes

is well established and in a 1 year of follow up study, a significant number of children changed their sub-type or outgrew their symptoms, indicating the instability of the proposed sub-types [12,40].

PREDISPOSING FACTORS FOR THE IBS

Numerous factors have been suggested as possible predisposing factors for IBS in children. However, pinpointing exact predisposing factors and avoiding them is a major challenge in the management of this condition.

Sex

Studies conducted so far have not shown gender as a clear risk factor for development of IBS. Some studies have reported a high prevalence of IBS in girls[2,11], while others failed to show such a difference[41]. Some studies have suggested that fluctuations in ovarian hormones may have an effect on development of symptoms in IBS[42,43]. However, the exact role of sex hormones on IBS is not yet clear.

Age

Many studies have reported higher prevalence of IBS in children between 8-12 years and decrease in prevalence with advancing age[2,4,11,41]. The decreased rates of prevalence with age are most likely due to spontaneous resolution of IBS with time.

Psychological factors

Several psychological factors have been recognized as risk factors for development of IBS. They include psychological stress[11,44,45], excess worry[45], anxiety[1,41], depression[1], physical, emotional and sexual abuse[46] and abnormal personality traits[1,10]. Furthermore, the adult studies have shown that these exposures lead to persistence of symptoms of FAPDs such as IBS into adulthood[47].

Early life events

Exact relationship between early life events and paediatric IBS is not clear. Some studies have reported high prevalence of FAPDs in patients exposed to gastric suction during neonatal period[48], born to mothers with gestational diabetes and pregnancy induced hypertension[49], admitted to a special care baby unit[49], having an umbilical hernia[50], having pyloric stenosis[51], with Henoch-Schonlein purpura[52] and with a history of cow's milk allergy[53]. However, further studies are needed to confirm these associations with IBS.

Gastrointestinal infections

Past history of gastroenteritis is a well-recognized predisposing factor for development of IBS in children and adults[2,41,54,55]. A meta-analysis has reported a mean prevalence rate of IBS as 9.8% in individuals with history of infectious gastroenteritis, while it was 1.2% in controls[56]. In another systematic review, pooled incidence of IBS is 10% following acute gastroenteritis[57]. Even higher incidence of IBS (10%-15%) has been reported after bacterial gastroenteritis[58]. The gastrointestinal infections commonly associated with post-infectious IBS are *Campylobacter species, Escherichia coli and Salmonella* species[54,55]. Studies conducted so far have failed to demonstrate a clear association between viral gastroenteritis and IBS[59].

Asthma and atopic disorders

IBS and asthma-related symptoms occur frequently together and are independently associated with each other in adults[60-65]. Similarly other studies have reported an increased risk of IBS in children with allergic diseases[66]. In contrast, some other studies have failed to find an association between asthma and IBS[67].

Diet

Gastrointestinal disorders such as IBS are commonly attributed to ingestion of different food items such as certain carbohydrates and fats[68,69]. There is some evidence that higher intake of spicy food and fried food increase the risk of IBS[1,70]. However, the exact relationship between IBS and diet is not clear. The exact role of food allergy in the development of IBS is not well researched either[71]. There is some evidence that food allergy is also associated with IBS in children[41]. In contrast some other adult studies failed to demonstrate such an association[62]. However, a significant percentage of patients with IBS have a restricted diet with exclusion of certain foods and drinks[69,72,73].

Socioeconomic, family and environmental factors

Few studies have reported an association between socioeconomic status and IBS[2]. Affluent social class[74] and exposure to cold are reported to be associated with this condition[1].

PATHO-PHYSIOLOGICAL MECHANISMS FOR IBS

Out of all the FGIDs, perhaps IBS is the most researched diseases entity in terms of its patho-physiology. These studies have reported large number of different and possible patho-physiological mechanisms. However, at the moment all these studies have only possibly touched on the surface of this complicated disease entity and have failed to demonstrate an exact patho-physiological mechanism/s. The majority of the patho-physiological studies include small samples, sometimes do not included a control group and the reported mean differences are too small to provide a statistical power to obtain meaningful conclusions[75]. In addition, some of the studies were conducted in a heterogeneous group of patients with chronic functional abdominal pain rather than in specifically in those with IBS and

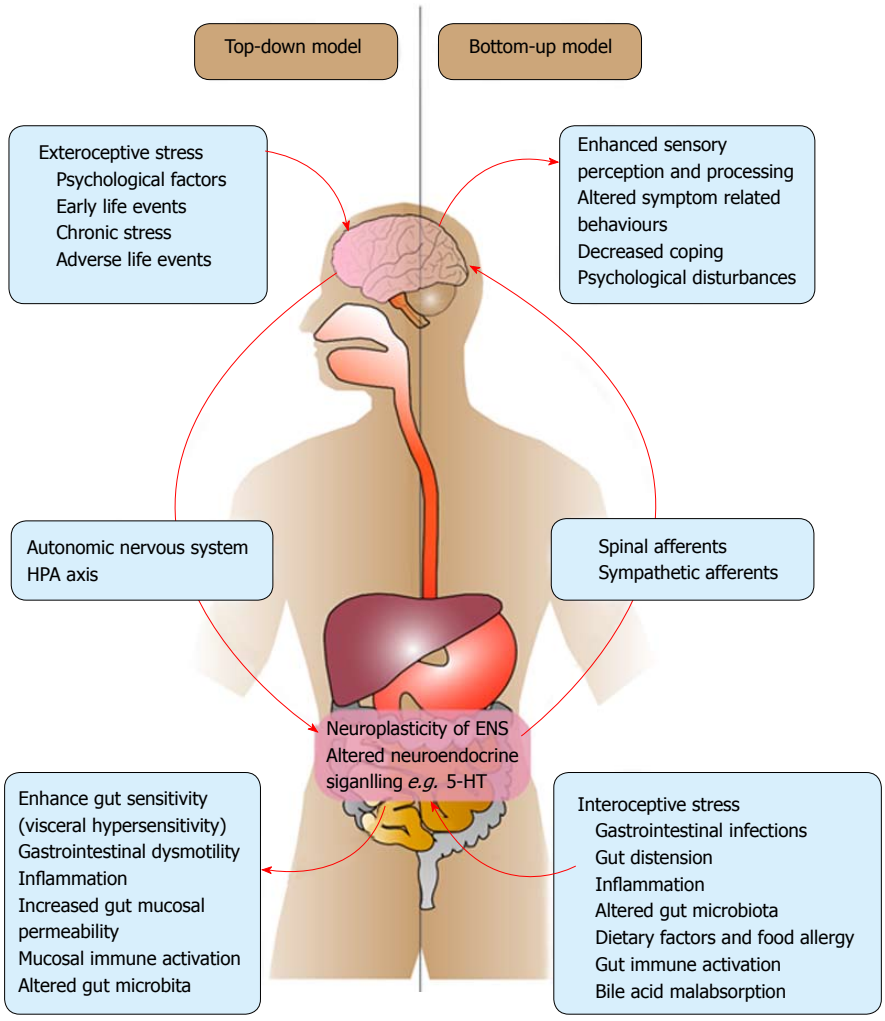

Figure 1 Top-down and bottom-up models of patho-physiology of irritable bowel syndrome. ENS: Enteric nervous system; 5-HT: 5-hydroxytryptamin; HPA axis: Hypothalamo-pituitary-adrenal axis.

therefore could not be exactly applied to IBS. Several controversies exist in the proposed patho-physiology of IBS[76]. Strangely, most of the proposed mechanisms do not correlate with the clinical symptoms.

Two models have been proposed to explain the patho-physiology of IBS[77]. First is the "top-down model" which suggest that the main patho-physiological changes are initiated in the brain. According to this model, the primary disease processes in the brain interact with the peripheral organs through the brain-gut axis to generate alterations in the gut leading to clinical expression of IBS[78]. The proponents of the "bottom-up model" which proposes that peripheral factors in the gut play the a key role and the alteration in cerebral functions are secondary to brain-gut interactions[79].

In both models, a large number of patho-physiological mechanism have been suggested and interactions between these mechanisms are believed to result in the development of IBS in susceptible individuals. Main suggested patho-physiological mechanisms for IBS are illustrated in Figure 1 according to the top-down and bottom-up models.

Top-down model vs bottom-up model

In top-down model, the symptoms of IBS are believed to be caused by alternations in the central nervous system initiated by various stressors directed at the central nervous system (exteroceptive stress) such as adverse life events[11,45,46]. anxiety[1,41] and depression[1]. It is believed that several neural networks of the brain interact with each other in an intricate manner to generate symptoms. Studies conducted in adult patients with IBS have reported interactions between central executive network (involving attention, working memory planning and response selection), salient network (responding to external and internal stimuli that reach to the brain), sensory motor network and autonomic networks (central control of autonomic function)[76,78,80]. These interactions are believed to alter the activity of the enteric nervous system through the autonomic nervous system and hypothalamo-pituitary-adrenal axis (HPA axis), causing physiological changes in the gut including visceral hypersensitivity and alteration in motility, permeability, secretion, immune reactions and the microbiome[78,80].

The bottom-up model suggests that various stressors directed at the gut can influence central nervous system and alter the cortical response to the visceral stimuli causing symptoms in IBS[77]. Intestinal infections, mucosal inflammation, gut distension, immune mediated reactions, food allergy, alterations in gut microbial flora, increased intestinal permeability and abnormal responses of the enteric nervous system to gut stimuli (*e.g.*, alterations in neurotransmitters such as serotonin) in combination or in isolation trigger symptom generation in this model. The gut may influence the brain via the intrinsic primary afferents neurons, whose cell bodies are located in cranial and dorsal nerve root ganglia. The sympathetic afferents from gut are believed to be the main mediator of nociceptive stimuli while vagal afferents are mainly believed to be involved in non-nociceptive sensations (*e.g.,* local reflexes, gastric accommodation *etc.*)[77].

The main problem is that the patho-physiological changes reported in the gut and the central nervous system in patients of IBS up to now can be attributed to both these models and identifying which comes first is like a chicken or the egg situation. However, the introduction of these two models has laid some foundations for direction of further research in the patho-physiology of IBS.

Communication between brain and the gut: brain-gut-axis

Both conceptual models recognize interactions between brain and the gut as the main patho-physiological mechanism in IBS[77]. This bidirectional communication is called as the brain-gut axis and consists of the central and autonomic nervous systems, enteric nervous system and neuro-endocrine system and the neuro-immune system[23,81].

Autonomic nervous system: Autonomic nervous system has been considered to be one of the main communicators between the brain and the gut[82] in both top-down and bottom-up models of pathogenesis of IBS. However, so far very few studies have been conducted to assess the autonomic nervous system in IBS and its exact role in generation of symptoms is not clear.

Studies conducted in adults have shown a correlation between vagal response and post-prandial abdominal symptoms of IBS-D and IBS-C[83]. Some other studies have reported abnormal gastric motility and underlying vagal defects[83,84]. Another study has reported abnormal fingertip blood flow responses in subjects with IBS suggesting excess sympathetic activity[85]. Findings of the above studies suggest that a shifting of sympathetic-parasympathetic balance may contributes to the pathogenesis of IBS. However, some other studies failed to demonstrate abnormalities in autonomic functions in patients with IBS[86].

However, all these studies have assessed either cardiovascular or ocular autonomic functions, but not the autonomic functions of the gut specifically. How these findings can be directly applied to the autonomic functions of the gastrointestinal tract is far from clear. Currently no exact technique is available to assess the gastrointestinal autonomic functions. Therefore, development of such a technique is a major challenge and will provide better opportunities to understand the role of the autonomic nervous system in gut functions in both health and disease.

Hypothalamo-pituitary-adrenal axis: The hypothalamic-pituitary-adrenal axis (HPA axis) is considered to be an important communicator in the brain-gut axis. HPA axis is activated by both exteroreceptive and interoceptive stress and therefore likely to be involved in both patho-physiological models. Activation of HPA axis ultimately results in increased release of corticotrophin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and cortisol. Increased release of CRH is believed to promote central sensitization while ACTH and cortisol tend to activate resident immune cells and extrinsic primary afferents in the gastrointestinal tract causing peripheral sensitization[23].

Corticotrophin releasing factor (CRF) is increasingly recognized as an important factor in the development of FGIDs including IBS. However, only a few human studies have been conducted so far and most of the assumptions are based on results of animal studies. One study reported an upregulation of CRF-Receptor type 1 (CRF-R1) in patients with IBS[87]. In addition, long-lasting epigenetic changes in the CRF expression have been reported in those exposed to neonatal stress, which results in the transcriptional responses to stress in adulthood[88]. In contrast, another study assessed the diurnal rhythm of cortisol and stress reactivity and showed that cortisol as a marker of stress does not have a major role in abdominal pain in infants[89]. Similar to humans, CRF-R1 upregulation, reversible mitochondrial damage and IBS like gut dysfunction were reported in rats after exposure to psychological stress. In this study, the increased CRF-R1 expression, reversible mucosal inflammation, increased epithelial permeability and conductance, and abnormal colonic response after exposure to stress lasted for a short duration (7 d) while visceral hypersensitivity observed after administration of exogenous CRF persisted for 30 d after exposure[90]. In agreement, others have reported that CRF and its receptors play an important role in stress related alterations of visceral sensitivity and gastrointestinal motility[91-93].

Observed patho-physiological changes in IBS

Numerous studies have been conducted in adults and children with IBS and large number of possible patho-physiological mechanisms have been suggested. However, they are like individual pieces of a large jig-saw puzzle and we are far from solving this complicated

problem. Fitting up available information on patho-physiology and finding the missing pieces of this puzzle is a major challenge.

Visceral hypersensitivity: Visceral hypersensitivity is defined as an enhanced perception of mechanical triggers applied to the bowel which seem as pain and discomfort[94]. In normal individuals, physiological changes in the gastrointestinal tract such as motility and distension do not cause pain. When there is altered sensory response to physiological stimuli, it is called visceral hypersensitivity. Two main types of visceral hypersensitivity have been identified so far. They are hyperalgesia and allodynia. Hyperalgesia is defined as in intensified pain sensation in response to normal stimuli which usually do not provoke pain, while allodynia is the elevated nociceptive sensation in response to normal stimuli[95].

Visceral hypersensitivity is considered to be the cornerstone in the patho-physiology of IBS[76]. One pediatric study has reported decreased rectal sensory threshold for pain in IBS and functional abdominal pain[96]. Another study in children with IBS has demonstrated that abdominal pain is associated with abnormal perception of visceral sensations and hypersensitivity[97]. Similar results have been reported in several other pediatric studies too[98-100]. Adults studies have also reported lowered rectal pain threshold in patients with IBS[101]. Several factors such as psychological stress, gastrointestinal infections, alterations in gut microbiota, inflammation, immunological factors, food, as well as genes, have been suggested to induce visceral hypersensitivity[98,102,103]. Visceral hypersensitivity is believed to be results from pain modulation at both peripheral level as well as at central nervous system[95].

Modulation of pain: (1) At the enteric nervous system: Main function of the enteric nervous system is to regulate local gastrointestinal reflexes and to transmit sensory information to the central nervous system for processing and integration[104]. A vast majority of afferent information received from the gut is used for regulation of normal functions such as motility and secretion[105]. Information regarding the sensory perception and modulation at the level of enteric nervous system is limited. One study assessing mast cell-induced excitation of visceral nociceptive sensory neurons in adults with IBS has suggested the possibility of initiation and perpetuation of symptoms through modulation of sensory neurons in the enteric nervous system[106]. (2) At the central nervous system: Increased pain perception in IBS is considered to be at least partly related to altered descending inhibition and pain affecting at the peripheral level and catastrophizing of pain at the central level[107]. It is reported that increased pain perception in IBS is not due to the tendency to report more pain but because of increased spinal nociceptive transmission[107] and impaired endogenous inhibition of somatic pain[108]. When functional magnetic resonance imaging (fMRI) was used, insular cortex and pre-frontal cortex are recognized as the main areas of the central nervous system which are involved in the processing of visceral pain in IBS[109]. It is also possible that alterations in pain appraisal, hypervigilance to interoceptive signals from the gut and engagement of emotional arousal could also contribute to the patho-physiology[110].

Alterations in neurotransmitters and receptors: More and more emerging evidence have recognized alterations in serotonin as important mediator in pathogenesis of IBS[111]. Serotonin (5-hydrodytryptamine; 5-HT) is an important neurotransmitter in enteric neurons and paracrine signaling substance secreted by enterochromaffin (EC) cells in the intestinal mucosa[112]. It mediates communication between the brain and the gut[95], and has been shown to be the responsible agent for bloating, nausea and vomiting[113,114]. In addition, it is considered to be an important signaling molecule in the central nervous system involved in mood, appetite, sleep, memory and learning. Alterations in serotonin is implicated in central nervous system disorders such as anxiety, depression and some psychiatric disorders[115]. Serotonin is removed by a highly selective transporter called the serotonin transporter (SERT). Gene polymorphisms of SERT receptors have been shown to be associated with IBS[116-118]. In addition, some distinct changes in EC cell numbers and content as well as release and uptake of serotonin appear to have relevance to the patho-physiology of IBS[115,119,120].

Gastrointestinal dysmotility: A large number of studies have demonstrated abnormalities in gastric myoelectrical activity[121-124], gastric motility[123-130] and accommodation[131,132] and intestinal and colonic transit[125,133-135] in patients with IBS and other FAPDs. Few have reported an association between motility abnormalities and exposure to stress[128]. It is suggested that stress can lead to alterations in central aminergic network involving serotonin and noradrenaline[136] and therefore believed to play an important role in the pathogenesis of IBS, especially in the top-down model. However, so far no clear relationship has been demonstrated between motility abnormalities and symptoms in children with IBS[128]. Therefore, whether the observed gastrointestinal motor abnormities are a cause for IBS or an effect of IBS is yet to be determined.

Immune mediated mechanisms: Increased prevalence of allergies and atopic disorders including asthma have been shown in patients with IBS[137-139]. But the small number of research ventures conducted up to now with small sample sizes have failed to demonstrate an exact link with immunoglobulin E (IgE)[140,141]. Increased numbers of mass cells have been reported throughout the gastrointestinal tract in patients with IBS[102,142,143]. It is suggested that serotonin

is released during degranulation of these cells and stimulates peripheral nerves in the submucosa and increases visceral sensitivity.

Infection, inflammation and intestinal barrier functions: It is suggested that visceral hypersensitivity observed in patient with IBS can be secondary to the activation of immune cells and to the development of low-grade inflammation. Studies conducted in children with IBS have demonstrated an accumulation of inflammatory cells in the intestinal mucosa[144]. A previous study conducted in children with FAP or IBS has reported an increased gut permeability and low grade inflammation. It has also been shown that the low grade inflammation was related to the degree to which pain interfered with activities[145]. The increased permeability is attributed to the enlarged spaces between epithelial cells, cytoskeletal condensation, abnormal gene and protein expression in tight junction proteins of intestinal epithelial cells and reduction in the expression of occluding and zonula occludens protein 1[146,147]. Bacterial mediated and proteasome mediated alterations have also been suggested as possible triggers for low grade inflammation which ultimately leads to increase intestinal permeability ("leaky gut")[148].

IBS is common after gastroenteritis and it is often of the IBS-D type[149]. In post-infectious IBS, gastrointestinal infections are believed to stimulate the immune system causing low-grade inflammation[150]. Post-infectious IBS is associated with hyperplasia of EC cells, increased counts of neutrophils, mast cells and T cells in the colonic mucosa. It is believed that gastrointestinal infections stimulate the immune system causing low-grade inflammation leading to post-infectious IBS[150].

Microbiota: Gut microbiota is reported to be different in patients with IBS that in healthy individuals, with increased Firmicutes/Bacteroids ratio, increased relative abundance of fecal Ruminoccus torque like phenotypes and reduced bacterial diversity with increase in certain bacterial species (Enterobacteriaceae, Veillonella, Dorea) and reduction of other species (*Bifidobacterium, Collinsella, Clostridiales*)[110,151]. Children with IBS have significantly higher percentage of *Haemophilus parainfluenzae* in their gut[152-154]. Increasing visceral sensitivity, altered gastrointestinal transit and increase in permeability of the intestine is reported in experimental studies using germ free animals receiving gut microbiota of patients with IBS, indicating a potential pathogenic role of gut microbiota[155]. Some other studies have reported an association between differences in short chain fatty acid production by colonic bacteria and the development of symptoms in diarrhea predominant IBS[156]. Interactions between the gut microbiota and food (fermented protein products, generation of gases) are potential sources for cell damage, altered barrier function as well as symptoms such as bloating and distension[157]. In addition, gut microbiota may influence other patho-physiological factors such as intestinal permeability, brain function, enteric nervous system, gastrointestinal motility and visceral pain, contributing to the patho-physiology of FGIDs[151]. However, further studies are needed, especially in children, to confirm the role of gut microbiota in IBS.

Food: Even though children have identified a large number of food items which exacerbates their symptoms only a few have been reported to be associated with IBS. IBS has been shown to be associated with fermentable oligo-, di- and monosaccharides carbohydrates and polyols (FODMAPs)[158]. However, the exact relationship between lactose and fructose mal-digestion and IBS is not clear[159]. Its relationship with fiber is rather controversial[160,161].

Genetic, epigenetic and environmental factors: Previous studies have reported that those with a family history of IBS or other bowel symptoms are more likely develop IBS[41,162]. Similarly, twin studies have suggested that there is a higher concordance of occurrence of IBS in monozygotic twins than in dizygotic twins[163]. The concordance rate of IBS in monozygotic twins was 17.2% while that was 8.4% in dizygotic twins[163]. However, if genetic factors play a major role in development of IBS, the concordance rate in monozygotic twins needs to be much higher. Therefore, it is possible that social and environmental factors also play an important role in development of IBS, in addition to the genetic predisposition[163]. This finding is further strengthened by other studies which reported that parents of children with FAPDs have higher tendency to develop similar illnesses[37,164].

A large number of genetic polymorphisms were considered to be associated with IBS. However, overall there is limited evidence of a genetic association[165]. The most frequently studied genetic associations are related to the serotonergic system, including serotonin transporter (SERT) gene polymorphisms[116,117]. MicroRNAs considered to play a role in the pathogenesis of IBS through regulating serotonin reuptake transport expression[166] and single-nucleotide polymorphisms rs56109847 led to reduce microRNA binding and overexpression of the target gene in intestinal cells increasing IBS-D risk[167].

Other gene polymorphisms involved in IBS include mitochondrial DNA polymorphism[168], alpha 2 receptor gene C-1291G polymorphism[169], cytokine gene polymorphisms (*e.g.*, IL-10 and IL 12 C (-1188) A)[170,171] and tumor necrosing factor super family (TNFSF) 15 polymorphism[172-174].

However, identification of a single gene polymorphism in patient with IBS alone would not possibly explain the complex nature of this disease. It is known that epigenetic changes in the genome play a crucial role in pathogenesis of diseases. It is possible that environmental factors,

psychological stresses, exposure to child maltreatments and some of the Patho-physiological mechanisms interact with each other in a very intricate manner to alter epigenetic DNA (by DNA methylation, histone modification) and changes in micro-RNA which can alter the gene expression (inhibition of increase transcription) to produce IBS phenotype. However, further evidence needs to be generated in this vital area of association between epigenetic changes and IBS[175].

DIAGNOSIS

IBS is a clinical diagnosis. Therefore, a thorough clinical evaluation is the most important part in the diagnosis. The process includes a detailed clinical history, including the past medical history, drug history, social and psychological histories. In addition, the physical examination generally should not reveal abnormalities that could indicate the possibility of an organic disorder. The clinical history is aimed at eliciting criteria laid down by the Rome committee[18]. Therefore, the main components of the clinical history should include details about abdominal pain, relieving and aggravating nature of the pain related to bowel motions and details about stool patterns. The symptoms need to be recurrent and should be at least four time per month for a minimum of 2-mo duration. The sub-type of IBS depends on the presence of a particular stool pattern. Presence of hard stools > 25% of the time with loose watery stools < 25% of the time helps to diagnose IBS-C whereas the opposite denotes the diagnosis of IBS-D. Alteration of stool pattern between diarrhea and constipation over a period of time suggests IBS-M whereas when the stool pattern is not like any of these, it leads to the diagnosis of IBS-U[18] (Table 1). In addition, children with IBS are suffering from a large number of somatic symptoms and psychological problems including maladjustment, depression and anxiety[10,46]. Eliciting these in the history would also help in the diagnosis and long term management of these children.

The diagnosis of IBS heavily depends on the nature of the stools and therefore the assessment of stool pattern is a crucial factor in the diagnosis. The current gold standard to assess the nature of the stools is the Bristol Stool Form Scale which was developed primarily using adult subjects (BSFS)[176]. Nonetheless, the consistency of the stools described by the BSFS correlated with the whole gut transit time in children[177]. More recently, Lane and colleague have developed a modified BSFS for children, which has only 5 stool types and that form has been shown to be reliable in children. Further evaluation of this scale, which looks more child friendly, is needed before using it to assess children[178].

However, the Rome criteria for children are not validated through a standard process as there is no gold standard to compare with. The diagnostic utility of Rome criteria to detect IBS and other FAPDs had a sensitivity of 0.35, specificity of 0.6, with negative and positive predictive values of 0.71 and 0.24 respectively[179]. In addition, the subtypes of IBS are known to change from one type to another over a period of time both in adults and children, questioning the validity of subtypes[12,180]. In addition, a significant percentage of children who fulfilled the Rome criteria for functional abdominal pain disorders were found to have organic pathologies on endoscopic examination of the upper gastrointestinal tract[179,181]. Therefore, further validation and refinement of Rome criteria may be needed to improve its diagnostic utility. Perhaps it is imperative to validate and calculate the likelihood ratios of each symptom of new Rome Ⅳ criteria to improve their clinical utility and that endeavor would be a challenge in the years to come.

Alarm features

Red flag features are a group of symptoms and signs that could indicate underlying organic pathology in children with IBS. The red flag features are an important concept as adult guidelines state that a safe diagnosis of IBS can be made using symptom based criteria in the absence of red flag features[182]. The Rome Ⅳ committee for childhood FGIDs has identified several features that should be considered as alarm features that indicate the potential of having organic diseases (Table 2)[18]. Clinicians are supposed to look for these features during the clinical evaluation mainly to rule out organic diseases such as inflammatory bowel disease and other disease entities that lead to malabsorption and growth failure. A study involving 606 patients (128 children with Crohn's disease and 478 with abdominal pain) found presence of anemia, blood in stools and weight loss are most predictive of having Crohn's disease[183]. Tam *et al*[184] studied 80 patients fulfilling Rome Ⅲ criteria for FD. They consider several alarm features including gastrointestinal blood loss, dysphagia, and persistent vomiting. After thorough investigations, including upper gastro-intestinal endoscopies in all these children, the positive and negative predictive value of the presence of alarm symptoms to detect organic pathology were 0.33 and 0.97 respectively[184]. Several other studies have also been conducted to assess the validity of the presence of alarm features in detecting organic disorders in children with recurrent abdominal pain[179,184-186]. However, since these studies have used a variety of symptoms and signs as red flag features it is extremely difficult to develop a validated set of red flag features to use in the day-to-day clinical practice to differentiate organic disorders from IBS. However, it is very valuable to have a set of alarm features to guide the clinician to decide on especially the invasive investigations such as gastrointestinal endoscopies, transit studies and other intrusive radiological procedures. One of the greatest challenges that we have is to develop a correct set of red flag features that have reasonable validity. Once available, these clinical clues could be used as a screening tool to rule out organic disorders and strengthen the clinical

Table 2 Potential alarm features in children with irritable bowel syndrome[18]

Family history of inflammatory bowel disease, celiac disease or peptic ulcer disease
Persistent right upper or lower abdominal pain
Dysphagia
Odynophagia
Persistent vomiting
Gastrointestinal blood loss
Nocturnal diarrhea
Arthritis
Perirectal disease
Involuntary weight loss
Deceleration of linear growth
Delayed puberty
Unexplained fever

diagnosis of IBS based on Rome symptom based criteria. This would undoubtedly minimize subjecting children to unnecessary and invasive investigations. It would be reasonable to use the following symptoms and signs as alarm features (Unintentional weight loss, significant lower gastrointestinal bleeding in the absence of an anal fissure, significant arthritis, any amount of upper gastrointestinal blood loss, persistent fever with abdominal pain, persistent diarrhea, family history of inflammatory bowel disease or celiac disease).

INVESTIGATIONS

It is generally considered that the diagnosis of IBS is solely dependent on fulfilling Rome criteria for children. The current concept is to make a clinical diagnosis using the latest Rome criteria and conduct a set of basic investigations to rule out common organic disorders. However, evidence is emerging that it is not solely a disease where there are no diagnostic biomarkers and thorough investigations would reveal significant pathologies in children who even fulfill Rome criteria for IBS. In this section we will discuss the important investigations that are useful in children with IBS.

Routine investigations

Most clinicians order routine investigations including blood count, inflammatory markers, routine biochemistry, urine microscopy, Celiac screening and ultra-sonogram to evaluate children with features of IBS. In a retrospective study, Dhroove and co-workers noted the low yield of commonly conducted blood, urine and stool tests to distinguish organic disorders from FAPDs[187]. In addition, these tests incurred a large sum of money. However, it could also possible that Dhroove and colleagues have selected a well-defined set of patients who have no organic disorders and therefore essentially bound to have no abnormalities in the investigation panel. Furthermore, no studies have assessed the diagnostic utility of these tests in FAPD. Therefore, the value of basic laboratory investigations in the diagnosis of IBS remains questionable. Furthermore, the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHN) committee on chronic abdominal pain also does not recommend performing these investigations in children[188]. It is reassuring for the practicing clinicians to conduct a few basic tests to rule out possible organic diseases that could present with recurrent abdominal pain and strengthen the diagnosis of IBS. This is also an opportunity to convince demanding parents that there are no dangerous organic disorders in the child and at the same time not subjecting them to unnecessary invasive investigations. However, yet again it is a challenge to develop a set of investigations that could help the clinician and the family while not subjecting children to unnecessary invasive procedures as data on this important aspect is sparse. A prospective study selecting children from a broader base rather than fulfilling Rome criteria for IBS and investigating for potential organic diseases and the use of Rome criteria to define IBS would be a useful way of addressing this issue. It is essential to consider the regional differences in the organic pathologies in the different parts of the world when compiling this list of investigations.

Coeliac screening

Coeliac disease (CD) is a multisystem disease and the gastrointestinal symptoms are similar to IBS with diarrhea. It is well known that gluten is a potential precipitating factor in a subset of patients with IBS even when the serology is negative (non-coeliac gluten sensitivity). A meta-analysis of adult studies has clearly demonstrated that pooled odds ratio for positive IgA anti-gliadin antibodies, endomysial antibodies and/or anti-tissue transglutaminase antibodies, and biopsy-proven CD in IBS subjects against controls were 3.21 (95%CI: 1.55-6.65), 2.75 (95% CI 1.35-5.61), and 4.48 (95%CI: 2.33-8.60), respectively[189]. On par with those results, the Turkish Celiac Study Group has found a borderline significant association between IBS and CD[190]. Furthermore, a study from Iran reported a higher prevalence of CD in children with recurrent abdominal pain compared to the general population[191]. Therefore, it could be recommended to screen children with IBS for celiac disease in areas known to have a high prevalence of that disease. However, prevalence of CD is increasingly noted in other parts of the world. CD is increasingly identified outside the Western world. Prevalence of 1% in children attending a tertiary care hospital was reported from North India[192]. Therefore, screening children present with symptoms of IBS (especially with IBS-D) for celiac disease would be an important step towards early recognition of CD in the future.

Fecal calprotectin

Calprotectin is a calcium binding protein that accounts for 60% of the protein in the cytosol of human neutrophils. Elevated calprotectin in feces indicates ongoing

neutrophil recruitment due to inflammation. Therefore, estimation of fecal calprotectin is increasingly used as a non-invasive screening for intestinal inflammation. Most pediatric gastroenterologists performed fecal calprotectin assay to rule out the possibility of inflammatory bowel disease (IBD). However, it should be noted that there is a variability of test results depending on the manufacturer[193]. A mild rise (< 50) or positivity of fecal calprotectin does not indicate the possibility of having IBD. It has been shown that children with IBD show a mean value of 349 μg/g whereas the majority of children with other diseases had a mean value of 16.5 μg/g[194]. Studying 126 children with pain predominant FGIDs, Flagstad and co-workers noted that the median calprotectin concentrations were at or lower than 16 mg/kg which was at the lower detection limit and there were no differences between the FGID subgroups. Nine children (7%) had slightly raised values[195]. All these results indicate the value of fecal calprotectin as a useful test in differentiating IBS from other inflammatory disorders presenting with a similar clinical picture.

Endoscopy

Endoscopy in pediatric gastroenterology practice is considered as an invasive procedure as it needs general anesthesia. Therefore, convincing parents for their child with features suggestive of IBS to undergo an upper or lower gastrointestinal endoscopy is a challenge to a pediatric gastroenterologist. Previous researchers have found significant number of disease entities when they performed endoscopies in children with recurrent abdominal pain, IBS or FD. Two studies from the Western world have noted 30%-37% of children with chronic abdominal pain and FAPDs had organic pathology such as *Helicobacter pylori* infection, peptic ulcer disease, inflammatory bowel disease, celiac disease and eosinophilic gastroenteritis to explain their symptoms. In both these studies presence of alarm symptoms did not predict the possibility of an organic disorder[181,186]. However, others have reported very low yield from the endoscopy and they found a changing number of red flag features that could be present in children with organic disorders[184,196]. Obtaining a negative endoscopy in children with chronic abdominal pain including IBS does not improve the clinical outcome[197]. Therefore, it is challenging to decide whether a child with IBS needs routine endoscopy or not. A great deal of clinical competence is needed to decide on this matter rather than depending on individual research findings and guidelines. Disease entities such as celiac disease, peptic ulcer disease, food allergy, and inflammatory bowel disease, including microscopic colitis, could only be confirmed by endoscopic biopsies. Therefore, children with substantial evidence (growth faltering, blood stained stools, chronic diarrhea, hematemesis) suggestive of serious organic pathologies should undergo endoscopy despite fulfilling criteria for IBS. Most of these disorders present with chronic loose stools. Therefore, the clinician should have a lower threshold to perform endoscopies in children presenting with IBS-D when they have some concerns regarding the presence of an organic pathology.

Radiological investigations

Clinicians tend to order ultra-sonogram of abdomen to evaluate children with IBS. However, performing ultrasonography in the absence of clear cut jaundice, recurrent vomiting and significant urinary symptoms would not yield any added benefit in the search for another cause for abdominal pain in these children[198].

Gastrointestinal motility investigations

Motility studies would help in some instances where the diagnosis is not straight forward. Non-invasive ultrasonographic evaluation of the gastric emptying and antral motility is useful in assessing children with IBS as it had been shown that children with IBS do have abnormal gastric emptying and antral motility[128]. Lower rectal sensory threshold for pain had been a prominent feature of children with IBS[99]. Furthermore, children IBS were found to have lower rectal sensory threshold for pain than controls and children with organic disease with abdominal pain[96]. Although not conducted in day-to-day practice, incorporating them into the positive diagnosis of IBS in cases where the diagnosis of IBS is not entirely clear would enhance the overall clinical care of these children. With the evolution of high resolution manometry in children it would be possible to detect novel abnormalities which could help in both understanding the patho-physiology and management.

MANAGEMENT OF IBS

An effective physician-parent-patient relationship is a major component of effective management of IBS. Management of IBS involves counselling and parental education, pharmacological and non-pharmacological therapies (Table 3).

Counselling and parental education

Most parents are anxious to know what is the cause for the abdominal pain in their children. They are often worried about serious medical conditions including possibility of malignancies. Once evaluation is over it is imperative that the clinician explain the negative investigations means that the child does not have a serious organic disorder and is having IBS. At this stage the clinician also needs to explain that the functional nature of the disease using diagrams and illustrations. The relationship between the family and the clinician would not only yield a positive diagnosis but also a global improvement of symptoms[199].

Parental behavior often does not help in childhood FAPDs. The anxiety of the parents would reflect in symptom severity and negatively influence the treatment outcome in children[200]. In a randomized controlled

Table 3 Management options for children with irritable bowel syndrome

Counselling and explanation to parents/child
Control maternal response to child's pain
Pharmacological interventions
Gastroprokinetics (domperidone)
Antidepressants (amitriptyline, citalopram)
Acid suppressing agents (famotidine, omeprazole)
Antispasmodics (peppermint oil, mebavarine, dotavarine)
Antihistamines (cyproheptadine)
Antibiotics (rifaximin)
Psychological interventions
Guided imagery
Gut directed hypnotherapy
Cognitive behavioral therapy
Yoga therapy
Neuromodulation
Low FODMAP diet
Probiotics

trial Walker *et al*[201] have shown that parental attention increases the complaints of children with FAPDs. In fact, it is possible to reduce parental solicitous response by cognitive behavioral therapy[202]. It has been shown that on long term follow-up some of these children outgrow their symptoms only with reassurance and education[40]. In addition, it is also possible that the initial diagnosis may change into a different category[40].

Hence, the time invested in counseling and explanation is an important part in the management. The challenge is that most of the gastroenterology clinics are over burdened with children suffering from FGIDs and the counselling would be time consuming[203]. Preparation of educational material for them to refer in the form of booklets and materials in official web sites of the clinics, or forming patient groups under the guidance of the clinicians would be the way forward in the future. Therefore, spending time with explanation of the possibility of functional nature of the disorder will be a very effective method of managing children with FAPDs.

Pharmacological management of IBS

Although many clinicians rely on the pharmacological approach, the overall efficacy of pharmacological agents in IBS is low. Several systematic reviews have pointed out the lack of well-designed high quality clinical trials in this area and lack of therapeutic efficacy of these agents[204,205]. The other issue pertaining to the clinical trials in children with FAPDs is that almost all paediatric trials have lumped together all FAPDs rather than including one specific FAPD such as IBS or FD. One of the major challenges that has come out of this practice is the inability to focus on one disease entity at a time. The small numbers of patient could be overcome by multicentre studies.

Current pharmacological agents to treat children with FAPDs include motility agents, antidepressants, acid suppressing agents, antispasmodics, antihistamines, and anti reflux agents. Most of these studies have included children with IBS under the umbrella term of recurrent abdominal pain and sub-group analysis has not been carried out to highlight the efficacy of the given therapeutic agent for IBS alone. This may partly be due to lack of statistical significance when analyzed according to the disease entities. In the following section the pharmacological and psychological interventions for children with FAPDs will be discussed, highlighting the efficacy of the intervention specifically of IBS when the data are available.

However, it is very difficult to prioritize and recommend one agent over the other in the background of lack of clear evidence and the choice depends upon several factors including availability, cost and the preference of the clinician. The latest guideline from the NASPGHN also does not recommend one agent over the other[188].

Gastroprokinetics: Gastroprokinetics are known to augment gastric motility and improve symptoms especially in adults with FD[206]. One double blind randomized placebo controlled clinical trial has evaluated the clinical efficacy of domperidone in children with FAPDs. In this study a significant cure rates, improvement of the overall clinical condition and reduction in pain severity was noted in the intervention group. The subgroup analysis pointed out that children with FAP-NOS respond better than IBS and FD. However, the clinical improvement had no relationship to the improvement of gastric motility. Although there were concerns regarding cardiac arrhythmias, no adverse reactions were reported during the treatment period[207].

Antidepressants: The brain gut microbiota axis has been implicated in the pathogenesis of FAPDs[208]. It is also well known that various centers and networks in the brain such as salient network, central executive network and sensory motor network have a combined output through autonomic network and the HPA axis, altering motility, secretions and microbiota in IBS[78]. Modulating these mechanisms in order to relive symptoms, tricyclic antidepressants and selective serotonin reuptake inhibitors have been used in clinical trials. However, in one well designed trial, amitriptyline was not superior to the placebo in relieving symptoms in children[209]. Another study with lesser methodological rigor found a significant improvement in health related quality of life in the treatment group[210]. Although there is a theoretical possibility of cardiac arrhythmias, both studies noted no adverse events related to treatment. Similarly, serotonin reuptake inhibitor citalopram has not shown any therapeutic benefit in children with FAPDs[211]. These findings are surprising as studies among adult patients with IBS showed a clear benefit of antidepressants therapy[212].

Although, the evidence is still lacking it is possible to use these drugs in a situation where the child is having

central symptoms such as anxiety and depression with abdominal pain. With available evidence, one of the future opportunities is to conduct a randomized trial using children with IBS alone.

Acid suppressing agents: Acid suppressing therapies are commonly used in children with FAPD, thinking that this is due to "gastritis" and suppressing acid in the stomach would reduce the symptoms. In an observational study of 290 children with chronic abdominal pain it was noted 2/3 of the patients were treated with proton pump inhibitors with a mean duration of 11 wk[181]. In other instances, simple H_2 receptor blockers are used by the clinicians. However, the evidence for this practice is lacking. In a randomized trial, although famotidine was noted to be superior to placebo in global symptom improvement, the drug fails to reduce abdominal pain[213]. Recent literature concerning adverse effects of long term acid suppression shows significant increase in respiratory and gastrointestinal infections, hypomagnesaemia, vitamin B12 deficiency and increase pathological fractures. In addition, it is worth to remember that it is possible to have rebound hyperacidity after discontinuation of therapy[214]. Therefore, long term acid suppression should be discouraged in children with FAPDs without proven *Helicobacter pylori* infection. However, this poses a big challenge as a large proportion of practitioners are used to prescribe acid suppression therapy for abdominal pain for generations.

Antispasmodic agents: Antispsmodic agents are known to reduce smooth muscle spasms in the gastrointestinal tract and are thought to reduce symptoms of FAPDs. One such agent is peppermint oil. Studies in adults have shown beneficial effects of peppermint oil in treating patients with IBS[215]. Two trials have been conducted in children with FAPDs testing the therapeutic benefits of peppermint oil[216,217]. Both have methodological flaws and analysis even after combining both studies. The evidence is still insufficient to recommend peppermint oil for the treatment of FAPDs[218].

Mebevarine is an antispasmodic drug which has direct effect on gastrointestinal smooth muscles[219]. It' s efficacy in treating children with FAPDs was assessed in a randomized controlled trial. After 4 and 12 wk of treatment with mebevarine, the treatment group had no superior reduction of pain compared to the placebo group [220].

In another randomized controlled trial, the efficacy of dotavarine was evaluated using 132 children with recurrent abdominal pain. Although the drug reduced the abdominal pain episodes and reduced missed school days when compared to the placebo group, the number of pain-free days after treatment did not differ significantly between both groups[221].

A Cochrane review has clearly indicated the potential benefits of antispasmodic treatment in adult patients with IBS[161]. However, those drugs such as cimetropium, dicyclomine, pinaverum and trimebutine have not been used in clinical trials of children with IBS.

Antihistamines: Cyproheptadine is an antihistaminic agent that has been tested in a small double blind placebo controlled trial as a potential therapeutic modality for children with FAPDs but not specifically IBS alone. Although the authors reported a positive result on reduction of abdominal pain intensity and frequency, small sample size and non-validated assessment tools reduce the legitimacy of the data[222].

Antibiotics: Rifaximin is a semisynthetic poorly absorbed antimicrobial derivative of rifamycin. Rifaximin formulation contains an extra pyrido-imidazole ring to reduce systemic absorption that is less than 1% after oral administration. Rifaximin elicits its antimicrobial properties by binding to the beta-subunit of the bacterial DNA-dependent RNA polymerase and thus inhibiting bacterial RNA syn283 synthesis. It has been approved for the treatment of IBS-D in adults[204].

Two clinical trials have assessed the efficacy of rifaximin in children with FAPDs[223]. Both studies have used children with all FAPDs rather than IBS alone. It is surprising to observe this trend in both trials as the drug is only recommended in IBS-D in adults. One of the well-known trials in adults to assess the efficacy is the TARGET trial which has shown the efficacy of rifaximin in patients who do not have small intestinal bacterial overgrowth[224]. Both pediatric trials have used lactulose breath test to include children into these studies. Although small intestinal bacterial overgrowth (SIBO) is known to produce symptoms similar to IBS, it's not known to be associated with FD or FAP. It was noted in the double blind placebo controlled trial that 2 wk of rifaximin was not effective in controlling abdominal pain in children[223]. It was surprising to note that 42% of children with positive lactulose breath test (LBT) had FD or IBS where SIBO had not been implicated in the patho-physiology. The other trial is an open label trial including 50 children with FAPDs (15% FD, 30% FAP and 55% IBS)[225]. All underwent LBT and children with positive LBT were treated with rifaximin for 7 d and others remained untreated. LBT became normal in 64% and they had significant improvement of their abdominal pain, bloating, and flatulence. Children who had abnormal LBT after treatment showed no improvement of symptoms. There was no subgroup analysis with regards to IBS in both trials.

Psychological interventions for IBS

Guided imagery: Guided imagery provides a state of engagement in imagery and relaxation[226]. It is considered as an effective intervention in children with pain[227] and could be delivered through an audio recording. In a pilot study comparing guided imagery

with standard medical care to standard medical care alone, van Tilburg and co-workers showed that guided imagery is superior to standard medical care in children with FAPD. The results also found that treatment effects were sustained up to 6 mo[228]. Another study compared guided imagery with progressive muscle relaxation with simple breathing exercise. In this study children who received guided imagery with progressive muscle relaxation had greater reduction in the number of days with pain and days with missing activity. However, the follow-up data was available only for 2 mo[227]. Both studies indicate the possibility of using guided imagery as a potential therapeutic option for FAPDs.

Gut directed hypnotherapy: Abnormalities in the brain-gut axis have been implicated with the patho-physiology of FAPDs in children. In addition, abnormal motility in the gastrointestinal tract and the visceral hypersensitivity have also been considered as contributory factors[208]. Gut directed hypnotherapy is used to teach necessary hypnotic skills to control and normalize gut function through several steps[229]. The approach has shown a beneficial therapeutic effect on several studies conducted in adults with refractory IBS[230]. Vlieger and co-workers conducted a randomized trial comparing gut directed hypnotherapy with the standard medical care in children with IBS or functional abdominal pain. Compared to the standard medical care, hypnotherapy was superior in reducing pain scores. In addition, at one year follow up treatment success was noted in 85% of children received gut directed hypnotherapy and 25% of children received standard treatment indicating effectiveness of hypnotherapy in FAPDs[231]. The therapeutic response was sustained even after 4.8 years of follow up[231]. When compared, hypnotherapy delivered using a compact disc is not inferior to the hypnotherapy given by a trained therapist in reducing pain intensity and pain frequency scores at 8 wk, post treatment 6 and 12 mo in children with FAP or IBS. In the subgroup analysis, no indication was found for different treatment effects in children with IBS and FAP. However, the publication provided no direct statistical data on the efficacy of hypnotherapy on IBS alone[232].

Cognitive behavioral therapy (CBT): CBT aims to improve the child's mental health and coping strategies, specifically in helping them to understand the onset and progress of their abdominal pain. It then offers the child a strategy to help manage it, along with anxiety management and behavioral techniques[233]. A recent Cochrane review has analyzed 10 interventional studies using CBT for chronic abdominal pain in children. In this analysis it was shown that all studies had parental involvement with the child although the number of sessions could vary. Methods they used are also diverse, including teaching of coping and distraction strategies, teaching of relaxation techniques, identification and change of negative pain related thoughts and modifying family responses to illness behavior[233]. When the data were pooled, CBT had a significant degree of success compared to controls in the short term (< 3 mo) follow up. However, the effect was not sustainable at 6 mo and one year. The analysis also found no evidence that CBT is effective on pain intensity scores after the intervention. Therefore, the data is not very supportive of using CBT in managing children with FAPDs. No data on efficacy of CBT on IBS were included in the analysis.

Yoga therapy: Yoga techniques involve a series of physical exercises, breathing techniques, combined with meditation methods aimed to reduce anxiety, improve body tone and increase feelings of wellbeing. When used as a treatment for FAPDs, yoga therapy is thought to improve altered function of the brain-gut-microbiota axis. Up to now, 3 clinical trials have assessed the efficacy of yoga therapy for children with FAPDs compared to controls using standard medical care or in a waiting list. According to a meta-analysis from the Cochrane group, the results show no advantage of yoga therapy on pain intensity, pain frequency and functional disability[233]. The review does not provide details of the efficacy of yoga therapy on IBS in children.

Other interventions

Neuromodulation: Neuromodulation uses a transcutaneous electrical stimulation to stimulate local skin nerve fibers and autonomic nervous system specifically, the efferent parasympathetic outflow to the gastrointestinal tract augmenting gastrointestinal function. There is evidence to prove that delayed gastric emptying and altered antral motility play a major patho-physiological role in IBS[128]. Kovacic *et al*[234] have tested this concept to treat FAPD in a clinical trial including 115 adolescents. Compared to the sham control group, adolescents who received electrical stimulation had improvement of pain after 3 wk of therapy. Therefore, although promising, it is difficult to recommend neuromodulation therapy for children with FAPDs without further studies. One of the limitations in this study, as in most of the other studies, is that they have not specifically assessed the efficacy of neromodulation on IBS.

Diet: Dietary components had been thought to be involved in the pathogenesis of FAPDs. Most parents tend to point out certain dietary items as sometimes the cause and sometimes the aggravating factor for FAPDs[235]. Studies in adults have shown diet containing fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) tends to alter intestinal function and microbiota and contribute to the pathogenesis of IBS[79]. Chumpitazi and co-workers have investigated the value of low FODMAP diet in treating children with IBS. In this randomized cross

over trial, children were randomized to receive 48 h of either low FODMAP diet or typical American diet. The authors found that, compared to the baseline, children had fewer daily abdominal pain episodes during the low FODMAP diet and more while they were on a typical American diet[236]. Although the results are promising, it is necessary to know the long-term efficacy of the intervention, including potential effects on growth and nutrition of children, before recommending low FODMAP diet for childhood IBS.

Fiber: Fiber consumption is thought to be beneficial for intestinal health. There are 4 randomized trials assessing the benefit of different types of fiber supplements in children with FAPDs (*e.g.*, psyllium, glucomannan)[237-240]. Fiber supplementation is believed to be helpful to soften stools, enhance colonic transit and improve fecal output[160,241]. Various measures were used to assess the outcome (Faces pain scale-revised, Birmingham IBS symptom questionnaire, Eong-Baker faces pain rating scale). When all 4 studies were pooled there was no significant difference between the fiber group and the placebo group in improvement of pain (OR = 1.83, 95%CI: 0.92-3.65) and pain intensity (SMD = -1.24, 95%CI: -3.41 to 0.94)[242].

Probiotics: Altered microbiome has been suggested as a potential patho-physiological mechanism of IBS. Reduction in bifidobacteria, lactobacilli and increased ratio of Fermicuticus: Bacteroid ration were noted in patients with IBS[78]. In addition, children with IBS were noted to have greater percentage of proteobacteria[152]. Therefore, probiotics (live microorganisms that improve the balance of intestinal microbiome) have been used in the treatment of FAPDs in children. Three studies have evaluated the efficacy of probiotics in children with IBS. The first study conducted by Bauserman and Michail included 50 children randomized to receive Lactobacillus GG or a placebo. After 6 wk of therapy the authors found no difference between the intervention and the placebo groups in relation to relieving abdominal pain or other gastrointestinal symptoms[243]. The other study that assessed the efficacy of Lactobacillus GG in 52 children (randomized to receive either LGG or placebo) noted a reduction in pain during the follow up period of 4 wk. No improvement in stool scale was noted during the study period[244]. In addition, it was also shown that LGG is effective in reducing frequency and intensity of pain in children with IBS compared to functional abdominal pain[245]. Guandalini *et al*[246] studied the utility of VSL 3# (probiotic mixture) in treating children with IBS in a double blind randomized cross-over trial. The results were encouraging and noted that VSL 3# could significantly relieve overall symptoms, reduce abdominal pain and bloating. However, no improvement was noted in stool patterns. Newlove-Delgado *et al*[242] conducted a systematic review and the meta-analysis of the value of probiotics in treating children with recurrent abdominal pain. In this meta-analysis, they conducted a subgroup analysis of the efficacy of probiotics in IBS and concluded that probiotics are effective in treating children with IBS [pooled OR of 3.01 (95%CI: 1.77-5.13; $P < 0.001$)], and the estimated number need to treat was four[242]. Therefore, probiotics can be recommended as a therapeutic modality for treating children with IBS. However, alteration of stool patterns (diarrhea, constipation) are equally disturbing to patients as well as abdominal pain. Therefore, it is imperative that the probiotic strains that could help in these aspects as well as abdominal pain should be used in future clinical trials. Developing a simple method available at the grass root level to identifying the microbial signature of children with IBS would be very useful as the clinicians can then individualize the probiotic treatment depending on the nature of the gut microbiome.

When consider the summarized evidence it is difficult to recommend one treatment over the other in treating children with FAPDs. It is mainly due to the small number of studies that have been conducted on most of the therapeutic modalities. Available guideline from the North American and European societies of Pediatric Gastroenterology Hepatology and Nutrition for managing children with chronic abdominal pain also does not recommend a clear pathway for using these therapeutic options[188].

However, it is possible to draw some tentative conclusions considering the available evidence. It is very clear that the long term use of acid suppressing agents could cause more harm than benefits and therefore, should be avoided in treating FAPDs. Similarly, encouraging an increase in the fiber content in the diet over the recommended amount (age in years + 5 in grams) is not helpful in the management of these children.

PREVENTION

IBS in children is a disease with substantial burden on healthcare systems and the economy. The cost of evaluation and in-patient care of children with IBS was rising from 1997 to 2009 in the United States (from USD 5278 to 18853)[15]. The estimated annual cost of caring for children with FAP/IBS in the European union is likely to be over 15 billion euros[16]. It has also been shown that parental productivity loss accounts for at least 22% of this cost. In addition, IBS also negatively affects the health related quality of life (HRQoL) of children. Several studies have shown the poor HRQoL in children with IBS[9,14,247]. Sagawa *et al*[13] have clearly illustrated the negative effect of IBS on the quality of school life in children. In addition, the current therapeutic armory is not adequate as most of the treatment for IBS in children are not evidence based. Therefore, it is of paramount importance to plan a preventive strategy to overcome the current challenges.

It has been shown that other functional gastroin-

testinal disorders such as infantile colic is preventable by prescribing prophylactic probiotic strains to patients who are vulnerable to develop it[248]. In addition, oral administration of Lactobacillus GG to neonatal rats following exposure to intracolonic chemical irritant did not develop visceral hyperalgesia later in the life and less biogenic amines and neurotransmitters involved in pain modulation[249]. Very similarly future research should aim to prevent development of IBS and other similar pain related FGIDs in vulnerable groups such as neonates exposed to interventions by using prophylactic therapeutic interventions.

It is known that exposure to abuse and other adverse life events predispose children to develop IBS and the severity of symptoms are related to the exposure[46]. Studies among adults have plainly illustrated that childhood exposure to abuse increase vulnerability to develop IBS in adults as well[250]. Preventive strategies implemented effectively with education about child protection and law enforcement against child maltreatment would be effective in preventing children being exposed to child abuse. When exposed, proper and prompt rehabilitation of these children will also be helpful in preventing development of IBS. Psychological stress is a key driving force in IBS. It is well known that exposure to stressful home or school related stressful life events predispose children to develop FGIDs including IBS[7]. Therefore, minimizing school related stress by more child friendly curricula and providing diverse educational opportunities for children would be an investment for the future. In addition, educating parents to minimize home related stresses such as alcoholism, frequent punishments etc., which would predispose children to develop IBS and related disorders would be another preventive step.

Exposure to gastrointestinal infection is another well known etiological factor especially in IBS[55]. Ensuring safe water, immunization against gastrointestinal infections and prompt treatment for infective diarrhea to minimize gastrointestinal inflammation need to be ensured across the communities where infections are common.

Attempts at prevention of IBS is a challenge and a future opportunity to reduce the disease burden and minimize the wastage of large sums of public funds. It has been shown that a significant proportion of adults with IBS had childhood chronic abdominal pain and possibly IBS[251]. Therefore, the attempt to prevent FAPDs including IBS in childhood possibly have a compound effect to minimize economic burden of adults with IBS as well.

CHALLENGES AND WAY FORWARD IN CHILDHOOD IBS

Epidemiology of pediatric IBS is well researched, but little is known of its patho-physiology and management. Most of the available evidence in this area is based on results of adult studies and how much these finding can be applied to childhood IBS is not clear.

Challenges and way forward in diagnosis of IBS

When it comes to IBS, applying the symptom based diagnostic criteria have become a challenge due to differences in interpretation of them between different regions in the world and different cultures. There are some attempts taken to understand cross cultural differences in reporting gastrointestinal symptoms and to overcome this problem.

Challenges and way forward in understanding patho-physiology of IBS

Patho-physiology is another main grey area in childhood IBS. Even though a lot of mechanisms have been suggested, their exact role in generation of symptoms is not clear. Most of the proposed patho-physiological mechanisms are heavily based on assumed theories rather than exact scientific evidence.

Up to now, almost all researchers and research groups have worked in isolation focusing on a single patho-physiological mechanism that could lead to IBS. However, the multi-factorial nature of IBS cannot be described using a single patho-physiological mechanism. It is quite possible that multiple mechanisms involved in the pathogenesis of IBS in a given child, and these mechanisms are likely to be different from another depending on the sociocultural, genetic and epigenetic factors. Therefore, a multi-professional collaborative research involving researchers and clinicians who have expertise in molecular and cell biology, organ physiology, genetic and epigenetic mechanisms, gastrointestinal motility, modern culture independent microbiological techniques, and cutting edge neurosciences is the only way to solve the enigma of the patho-physiology of IBS in children. The proposed top-down and bottom-up models[77] have laid the foundation for this integrated approach to study and understand the patho-physiology of childhood IBS.

Challenges and the way forward in the management of IBS

It is difficult to overcome the therapeutic demand of childhood IBS using the same conventional therapeutic agents that had been used during the past decades. Most of these agents are not very promising in the current evidence-based era. There are a number of novel agents that pediatric researchers could use by learning from adult counterparts. Some of these agents are already approved by the drug regulatory authorities for adults. Therefore, using well characterized patients diagnosed based on current Rome Ⅳ criteria, the efficacy of the agents such as elobaxibat, lubiprostone, linaclotide, 5-HT 3 receptor antagonists such as alosetron and ondansetron need to be tested for treating children with IBS.

As mentioned earlier the patho-physiology IBS is multifactorial[78,79]. It is believed that more than one

patho-physiological mechanism might be operating in generating symptoms in one patient. Most of the drugs that are used in children have tried to address one patho-physiological mechanism in the belief that correction of one such mechanism would alleviate symptoms in a given patient. This approach is probably not the best way of dealing with a child with IBS in real life.

The main feature of children with IBS is pain in the abdomen. Therefore, it is essential to use a pain reliever initially. Depending on the type of pain the clinician could use a smooth muscle relaxant or a gastroprokinetic. In addition, if a child with IBS is having a lot of psychological disturbances including anxiety and somatization with constipation it is better to treat this child with a centrally acting management option (amitriptyline, gut directed hypnotherapy or cognitive behavioral therapy) with a laxative (polyethylene glycol, linocletide, lubiproston) to relieve symptom of constipation. Similarly, if the child is suffering from diarrhea predominant IBS one can approach with a probiotic or rifaximin to restore the gut flora. However, we need to generate evidence through well conducted focused studies using children with IBS alone.

In the recent past, more and more possible predisposing factors are recognized for IBS, including gastrointestinal infections, asthma and allergy, dietary factors and genetic and epigenetic factors. Therefore, the time has come to start focusing on prevention strategies targeting those patients with a high risk of developing IBS, in addition to management of affected individuals.

CONCLUSION

IBS is a common FGID among children around the world. A large number of children are suffering because of intestinal and extra-intestinal symptoms of IBS. However, little is known of its exact patho-physiology and management. Novel research using advanced technologies based on proposed top-down and bottom-up models of patho-physiology and treatment trials focusing on multiple combined interventions are likely to be more beneficial in understating and treating paediatric IBS. Many risk factors have been recognised for IBS. Therefore, the time has come to explore possible prevention strategies for this problem.

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REVIEW

Prognostic and predictive blood biomarkers in gastric cancer and the potential application of circulating tumor cells

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Abstract

Gastric cancer (GC), with its high incidence and mortality rates, is a highly fatal cancer that is common in East Asia particularly in China. Its recurrence and metastasis are the main causes of its poor prognosis. Circulating tumor cells (CTCs) or other blood biomarkers that are released into the circulating blood stream by tumors are thought to play a crucial role in the recurrence and metastasis of gastric cancer. Therefore, the detection of CTCs and other blood biomarkers has an important clinical significance; in fact, they can help predict the prognosis, assess the staging, monitor the therapeutic effects and determine the drug susceptibility. Recent research has identified many blood biomarkers in GC, such as various serum proteins, autoantibodies against tumor associated antigens, and cell-free DNAs. The analysis of CTCs and circulating cell-free tumor DNA (ctDNA) in the peripheral blood of patients with gastric cancer is called as liquid biopsy. These blood biomarkers provide the disease status for individuals and have clinical meaning. In this review, we focus on the recent scientific advances regarding CTCs and other blood biomarkers, and discuss their origins and clinical meaning.

Key words: Gastric cancer; Biomarker; Circulating tumor cells; Autoantibodies; Cell-free DNA

Core tip: As liquid biopsy, the detection of circulating tumor cells (CTCs) and other blood biomarkers have their certain clinical significance. In this review, we focus on the recent scientific advances of CTCs and some other blood biomarkers, and discuss their origin and clinical usefulness.

Li TT, Liu H, Yu J, Shi GY, Zhao LY, Li GX. Prognostic and predictive blood biomarkers in gastric cancer and the potential application of circulating tumor cells. *World J Gastroenterol* 2018; 24(21): 2236-2246 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i21/2236.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i21.2236

INTRODUCTION

Gastric cancer (GC) ranks as the fifth most common malignant tumor and the third leading cause of cancer deaths, with more than 951000 new cases and 723000 deaths estimated per year (GLOBOCAN 2012)[1]. Despite the development of diagnostic techniques, surgical techniques and perioperative management in recent years, the prognostic outcomes for GC remain poor. Because early stage GC tends to be asymptomatic and because mass screening is not popular, most patients in China are diagnosed at an advanced stage[2]. The prognosis of peritoneal metastasis from gastric cancer is very poor. In addition, the median survival is 4-12 mo, and the 5-year actuarial survival rate of patients with peritoneal metastasis is less than 5%[3,4]. Therefore, finding useful diagnostic and monitoring tools for gastric cancer patients should be considered as the most important clinical objectives.

A "liquid biopsy" for gastric cancer patients is used to detect physiological indicators or parameters in the serum; the procedure is less invasive than an endoscopic or surgical biopsy, and it allows practitioners to detect the disease earlier and visualize the dynamics and development of gastric tumors, as well as treatment efficiency and chemotherapy resistance. Carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9) and cancer antigen 72-4 (CA72-4) are regarded as clinically popular gastrointestinal tumor biomarkers. However, their positivity rates are less than 40% in GC patients, and the sensitivity and specificity of these blood biomarkers are not sufficient[5,6]. Indeed, if a blood biomarker is to be used in a population-based screening program, it should be reliable in repeated applications and easily measurable in blood serum or plasma by common laboratory equipment. Moreover, it should be present in the bloodstream before the onset of manifestations and clinical symptoms, be able to distinguish between cancer and inflammation and have high positive predictive value for malignant tumors. Therefore, there is an urgent need to identify more precise and effective blood biomarkers to provide optimal management for GC patients; these blood biomarkers should be able to provide early detection, clinical staging, therapy response monitoring, and prognosis for GC.

Cells can be released into the blood stream from the original tumor and/or corresponding distant metastatic sites. These circulating tumor cells (CTCs) could be collected and detected through respective technologies according to their physical and biologic features. CTCs from cancer patients may be considered as a type of real-time "liquid biopsy" that could provide real-time information about the cancer status. CTCs have already been accepted by the FDA as a prognostic biomarker for monitoring patients with breast, prostate and colorectal cancer[7]. Currently, the concept of "liquid biopsy" has also been accepted for the clinical application of evaluating ctDNAs that apoptotic and necrotic cancer cells discharge into the blood circulation[8]. As we know, there are numerous genetic and epigenetic aberrations that could activate oncogenes and promote tumor progression. Therefore, we have developed sensitive molecular assays for the detection of ctDNAs in the blood plasma to find tumor-specific aberrations. Moreover, several autoantibodies against specific tumor associated antigens (TAAs) that are expressed by cancer cells and can be detected in the blood plasma more than five years prior to diagnosis have already been identified[9]. Therefore, CTC, ctDNAs and autoantibodies could become potential blood biomarkers for gastric cancer[10].

In this article, we focus on the clinical applications of CTC, ctDNAs and autoantibodies after a brief introduction of the biology and detection technologies, and we explore the future prospects of blood biomarkers in gastric cancer patients.

THE BIOLOGY BEHIND CTCs

The cancer cells that are released from the original tumor or corresponding distant metastatic sites into the circulating blood are called CTCs. However, these epithelial tumor cells cannot stay in the harsh conditions of the bloodstream, and it is possible that CTCs are selected through these harsh conditions[11]. This proposal is consistent with the phenomenon that there are many apoptotic or fragmented CTCs in the peripheral blood stream of cancer patients[12]. The treacherous journey through the vasculature is necessary for the spread of cancer cells to additional sites. CTCs are closely associated with activated platelets and macrophages[13]. Moreover, the transference of metastatic cancer cells into the circulating blood often relies on various chemokines, such as CCR4, CCR7, CCR9, and CXCR4, which guide the cancer cells across the blood vessels[14]. Even a few months or years after primary tumor removal, CTCs can be detected in the peripheral bloodstream of cancer patients, which indicates that cancer cells can be released into the circulation from other metastatic sites[15,16]. However, how these CTCs give rise to tumor metastasis

and progression remains unclear. Future comparative genomic analyses of primary carcinoma and metastatic specimens along with CTCs from the same patient might provide more insight (Figure 1).

Currently, CTCs are often detected by epithelial markers such as epithelial cell adhesion molecule (EpCAM) and cytokeratins (CKs), which are not expressed on the surface of blood cells and distinguish CTCs from the masses of blood cells[17]. Epithelial cancer cells can make an epithelial-to-mesenchymal transition (EMT) that leads to decreased epithelial marker expression and enhanced plasticity and migration and invasion capacity. The CTCs that undergo EMT could be resistant to anoikis, which are necessary for the survival and dissemination of CTCs[17]. It has been previously indicated that EMT might particularly affect the stemness of tumor cells[18]. CTCs that undergo EMT might escape detection by EpCAM-based collection methods, such as the CellSearch system. Our previous study explored mesenchymal markers (Vimentin and Twist) to identify the mesenchymal phenotypes of CTCs in the bloodstream and their relevance to therapy responses[19].

TECHNOLOGIES FOR CTCs DETECTION

The evolution of various technologies to enrich and detect CTCs has been considerable, even resulting in the detection and verification of new CTC markers[17]. It is vital that we pay close attention to the biological characteristics of tumor cells dissemination and potential stem cell like properties that are affected by EMT, particularly in the field of CTCs[18]. Therefore, many companies have optimized their devices to select and detect CTCs that have undergone EMT[17].

After an enrichment step, we could greatly increase the concentration of CTCs and enable the easy detection of even a single tumor cell. Then, CTCs can be detected by different techniques. In theory, CTCs could be positively or negatively chosen based on physical features (*e.g.*, size, density, deforming character, and electric charges) and biologic features (*e.g.*, the expression of protein markers). The enrichment of positively or negatively chosen CTCs could also be achieved based on particular combinations of physical and biologic features in a device. Then, the CTCs could be detected through immunologic, molecular, and/or functional assays. Recently, increasing numbers of research teams have attempted functional tests using cultures and xenografts of CTCs[20,21]. *In vitro* and *in vivo* CTCs models can be applied to detect individualized drug susceptibility. However, the ability to establish CTCs cultures and xenografts of CTCs should be improved to design personalized medicine. Currently, hundreds or thousands of CTCs are required to construct cancer cell cultures or xenografts, which limits this approach to individual therapy (Figure 1).

The new technical developments that we focus on are based on new discoveries in CTC biology. A lack of knowledge has hindered the development of the application of CTCs for clinical diagnosis. However, new significant perspectives regarding the biological meaning of CTCs and various revolutionary techniques have been reported[22]. We believe that equipment for the combined collection, detection, and characterization of CTCs will soon be applied clinically.

CTCs AS AN INDICATOR FOR GC RECURRENCE AND METASTASIS

Recurrence and metastasis not only predict clinical outcomes but also affect the quality of life of GC patients. They are the most critical factors in the treatment of GC. It was originally thought that incomplete surgical resection resulted in recurrence and metastasis after the operative treatment of GC; therefore, extensive radical resection was applied. However, this procedure was not successful, indicating that there are other possible reasons for recurrence and metastasis. Some researchers found that tumor cells could be released into the bloodstream at the early stage of solid tumors (*e.g.*, breast, colon, lung, and gastric cancer)[7]. Therefore, CTCs may also play a vital role in monitoring the dissemination of gastric cancer and guiding the treatment of GC patients with recurrence and metastasis.

As summarized in Table 1, many studies have reported the clinical value of CTCs as prognostic indicators by different detection methods, including the CellSearch system, RT-PCR/qRT-PCR, and FISH. Uenosono *et al*[23] detected CTCs using the CellSearch system in 251 gastric cancer patients and found that the overall survival (OS) was obviously lower in patients with CTCs than in patients without CTCs ($P < 0.0001$). Subgroup analysis revealed that the relapse-free survival and OS were significantly lower in patients with CTCs than in patients without CTCs in the resection group ($P < 0.0001$). In a prospective study, Matsusaka *et al*[24] also assessed the correlation between CTCs detected by the CellSearch system and chemotherapy and clinical outcomes. They found that GC patients with at least 4 CTCs at 2 and 4 wk after the onset of chemotherapy had an obviously shorter overall survival and progression-free survival than the patients with less than 4 CTCs. However, the CTCs levels at baseline (*i.e.*, before chemotherapy) had no positive correlation with the clinical outcomes. These findings may indicate that the treatment response of CTCs is correlated with clinical outcomes. The number of studies using RT-PCR/qRT-PCR methods is relatively small. However, Mimori *et al*[25] detected a candidate marker, the membrane type 1 matrix metalloproteinase (MT1-MMP) mRNA level, in more than 800 GC patients. This marker was chosen based on the results of a cDNA microarray analysis, and its correlation with prognosis was subsequently validated using qRT-PCR. As a consequence, the MT1-MMP mRNA level in the peripheral blood may be an independent prognostic indicator of recurrence and metastasis in GC patients ($P = 0.0018$).

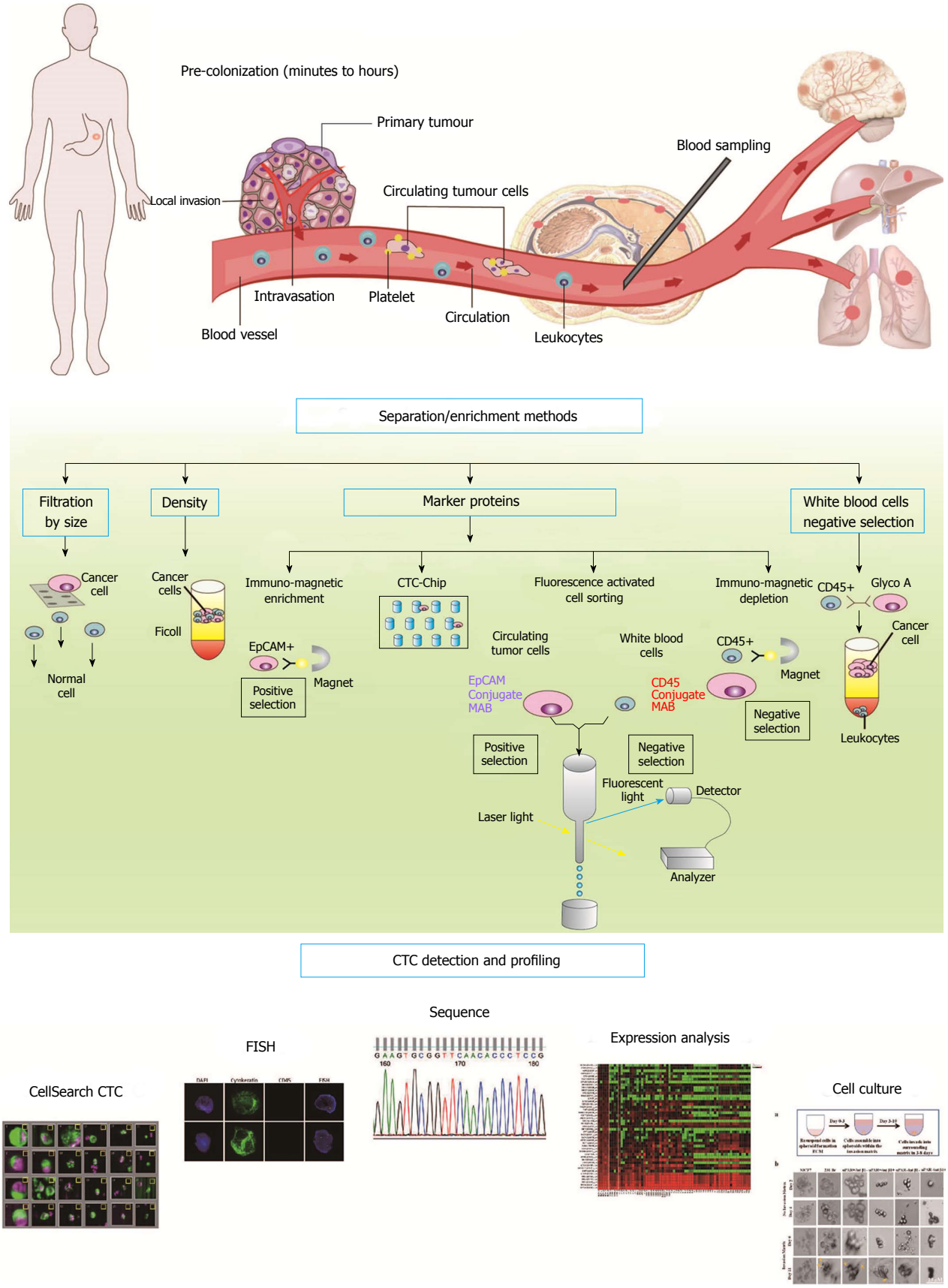

Figure 1 Flow chart of current and potential applications of circulating tumor cell. Circulating tumor cells (CTCs): The blood samples from cancer patients are processed through various isolation/enrichment and detection techniques. CTCs are usually captured along with contaminating leukocytes. Various detection methods are utilized to detect the rare cell population in the bloodstream.

Table 1 Prognostic value of circulating tumor cells in gastric cancer

Characteristic and number of patients		Detection method			Statistic value		Ref.
	17	RT-PCR	CA19 mRNA	OS	$P = 0.014$	CK19 (+) *vs* (-)	Yeh *et al*[44], 1998
I -IV	57	RT-PCR	CEA mRNA	Liver metastasis recurrence	$P = 0.03$	CEA (+) *vs* (-)	Miyazono *et al*[45], 2001
I -IV	106	RT-PCR	CEA mRNA	Recurrence/ metastasis	$P = 0.02$	CEA (+) *vs* (-)	Sumikura *et al*[46], 2003
I -IV	46	qRT-PCR	CK20 mRNA	2-yr-survival	$P < 0.05$	CK20 (+) *vs* (-)	Friederichs *et al*[47], 2005
I -IV	41	RT-PCR	CK20 mRNA	OS	$P = 0.0363$	CK20 (+) *vs* (-)	Illert *et al*[48], 2005
I -Ⅲ	46	RT-PCR	CEA mRNA	Recurrence	$P \leq 0.00022$	CEA after sugery (+) *vs* (-)	Seo *et al*[49], 2005
I -IV	52	RT-PCR	C-Met mRNA MUC1 mRNA	OS OS	$P = 0.0178$ $P = 0.0352$	C-Met (+) *vs* (-) MUC1 (+) *vs* (-)	Uen *et al*[50], 2006
I -IV	42	qRT-PCR	CEA mRNA	Recurrence/ metastasis	$P = 0.032$	CEA (+) *vs* (-)	Wu *et al*[51], 2006
I -IV	64	MAH	hTERT/CK19/CEA/ MUC1	Recurrence/ metastasis	$P = 0.009$	All marker (+) *vs* the others	Wu *et al*[52], 2006
I -IV	57	RT-PCR	CK20 mRNA	5-yr survival	$P > 0.05$	CK20 (+) *vs* (-)	Pituch-Noworolska *et al*[53], 2007
Metastatic	27	CellSearch System	EpCAM CK8/18/19	OS	$P = 0.039$	CTC ≥ 2 *vs* < 2	Hiraiwa *et al*[54], 2008
I -IV	69	RT-PCR	CK19 mRNA CK20 mRNA	OS OS	$P = 0.0347$ $P = 0.049$	CK19 (+) *vs* (-) CK20 (+) *vs* (-)	Koga *et al*[55], 2008
I -IV	810	RT-PCR	MT1-MMP	Recurrence/ metastasis	$P = 0.0018$	MT1-MMP (+) *vs* (-)	Mimori *et al*[25], 2008
I -IV	55	RT-PCR, ELISA	Survivin mRNA	RFS	$P = 0.026$	Survivin (+) *vs* (-)	Yie *et al*[56], 2008
I -IV	70	qRT-PCR	Survivin mRNA	OS	$P = 0.036$	Survivin high *vs* low	Bertazza *et al*[57], 2009
Advanced	51 (2 wk after chemotherapy) 48 (4 wk after chemotherapy)	CellSearch system	EpCAM CK8/18/19	PFS ,OS (2 wk after chemotherapy) PFS ,OS (4 wk after chemotherapy)	$P < 0.001$	CTC ≥ 4 *vs* < 4	Matsusaka *et al*[24], 2010
I -IV	123	qRT-PCR	CEA mRNA	Recurrence DFS	$P = 0.001$ $P = 0.001$	CEA (+) *vs* (-)	Qiu *et al*[58], 2010
I -IV	30	qRT-PCR	CK18 mRNA	RFS OS	$P < 0.001$ $P = 0.001$	CK18 (+) *vs* (-)	Saad *et al*[59], 2010
I -IV	95	qRT-PCR	B7-H3 mRNA	OS	$P = 0.046$	B7-H3 high *vs* low	Arigami *et al*[60], 2011
I -IV	98	RT-PCR, ELISA	Survivin mRNA	DFS	$P < 0.001$	Survivin (+) *vs* (-)	Cao *et al*[61], 2011
I -IV	52	qRT-PCR	miR-200c	OS RFS	$P = 0.016$ $P = 0.044$	miR-200c high *vs* low	Valladares-Ayerbes *et al*[62], 2012
I -IV	75	Immunofluorescence	GFP	OS	$P = 0.0021$	CTC ≥ 5 *vs* < 5	Ito *et al*[63], 2012
I -IV	251	CellSearch system	EpCAM CK8/18/19	OS RFS	$P < 0.001$ $P < 0.001$	CTC (+) *vs* (-)	Uenosono *et al*[23], 2013
I -IV	22	CellSearch system	EpCAM CK8/18/19	OS PFS	$P = 0.23$ $P = 0.91$	CTC ≥ 2 *vs* < 2	Sclafani *et al*[64], 2014
I -IV	62	qRT-PCR	KRT19/MUC1/ EPCAM/CEACAM5/ BIRC5 mRNA	OS PFS	$P = 0.003$ $P < 0.001$	All marker (+) *vs* the others	Kubisch *et al*[65], 2015
I -IV	36	Flow cytometry	CD133 ABCG2	OS	$P = 0.034$	CD133 (+) *vs* (-)	Xia *et al*[66], 2015
I -IV	136	CellSearch system	EpCAM CK8/18/19	PFS	$P = 0.016$	CTC (+) *vs* (-)	Okabe *et al*[67], 2015
I -IV	100	Cell Search system	EpCAM CK8/18/19	OS PFS	$P = 0.004$ $P = 0.004$	CTC ≥ 5 *vs* < 5	Lee *et al*[68], 2015
I -IV	24	FACS-ICC	EpCAM	OS PFS	$P = 0.014$ $P = 0.007$	CTC ≥ 2 *vs* < 2	Meulendijks *et al*[69], 2016
I -IV	136	CellSearch system	EpCAM CK8/18/19	OS PFS	$P < 0.001$ $P = 0.001$	CTC ≥ 3 *vs* < 3	Li *et al*[70], 2016
I -IV	65	Immunofluorescence	OBP-401	OS RFS	$P = 0.183$ $P = 0.034$	OBP-401 (+) *vs* (-)	Ito *et al*[71], 2016
I -IV	106	CellSearch system	EpCAM CK8/18/19	OS RFS	$P = 0.003$ $P = 0.0002$	CTC ≥ 2 *vs* < 2	Peront *et al*[72], 2017
I -IV	43	IsoFlux platform	EpCAM	OS	$P = 0.0013$	CTC ≥ 17 *vs* < 17	Brungs *et al*[73], 2018

qRT-PCR: Quantitative real-time polymerase chain reaction; MAH: Membrane-array hybridization; DFS: Disease-free survival; OS: Overall survival; PFS: Progression-free survival; RFS: Relapse-free survival.

Taken together, these studies indicate that CTCs result in GC recurrence and metastasis and may act as vital therapeutic targets for the treatment of GC recurrence and metastasis after radical resection.

OTHER POTENTIAL BLOOD BIOMARKERS

Cell-free nucleic acids

Tumor DNA can be released into the blood stream from the primary tumors, circulating tumor cells, or metastases of cancer patients. The majority of circulating cell-free tumor DNAs (ctDNAs) come from apoptotic or necrotic cancer cells that release fragmented DNA into the circulating blood. Dying nonmalignant host cells can also release cell-free DNAs (cfDNAs) into the circulating blood. These normal cfDNAs can dilute the ctDNAs concentrations in cancer patients, particularly in circumstances when tissue-damaging procedures, including surgery, chemotherapy, or radiotherapy, were carried out. Even though the length of DNA fragments might provide some information about the derivation of cfDNAs[26,27], we should further explore the biological features of the ctDNAs in the circulating blood. Several studies have indicated that ctDNAs can even be absorbed by host cells, and this uptake can affect the biology of these host cells[28,29]. Thus, ctDNAs may be indicated as a new target for anti-tumor treatment in order to dilute this type of oncogenic DNA, an idea proposed decades ago[30]. Several clinical applications of ctDNAs have been used for gastric cancer. ctDNAs is not only a tool for the early detection of cancer but also a prognostic or predictive factor (Table 2).

Among previous studies of ctDNAs in GC patients, we found that some studies focused on the concentration of ctDNAs. In these studies, the housekeeping gene, beta-actin[31], and a non-coding DNA sequence, ALU[32], were assessed. By comparison, the most widely used method for detecting ctDNAs is the measurement of methylated DNA in the plasma or serum; this measurement is often performed with methylation specific-PCR (MSP) or quantitative methylation specific-PCR (qMSP) assays. With the advances in technology and verification of more sensitive and specific genes, evidence has accumulated in this field. Comprehensive analyses using methylation CpG island microarray have indicated the possibility of more meaningful genes for measuring methylated DNA. Furthermore, Ling *et al*[33] have shown the effective application of methylated XAF1 DNA. This DNA could be used as a diagnostic or prognostic biomarker with high specificity and sensitivity. In addition to mutation analyses, gene amplification appears to provide relevant blood biomarkers. Park *et al*[34] found that the combination of plasma HER2 and MYC concentrations to diagnose GC had a sensitivity and specificity of 69% and 92%, respectively. To determine the effect of sequencing methods upon the overall diagnostic accuracy, Shoda *et al*[35] compared qPCR and digital droplet PCR (ddPCR)[36] for detecting the HER2 amplification ratio in 60 patients with GC. A correlation between the plasma and tissue HER2 amplification ratios was observed by ddPCR (ρ = 0.424, 95%CI: 0.125-0.652, P = 0.00721).

Cancer-associated autoantibodies

IgG Autoantibodies against specific tumor associated antigens (TAAs) can be detected in the blood more than five years prior to a clinical diagnosis of cancer, thus indicating their important role in the prognosis of early-stage cancer[37,38]. Additionally, autoantibodies have other promising biomarker qualities: they can be detected in every type of tumor that has ever been tested[39,40] and they are very stable and have antigen specificity. Assessing the autoantibody response against TAAs with multiplex immunoassays is supposed to be viable, and this method might make them clinically applicable.

To the best of our knowledge, ten studies have reported the clinical diagnosis values of diverse GC associated autoantibodies or their combinations (Table 3). In these studies, the recognized biomarkers can distinguish GC patients from healthy controls with comparatively excellent specificity (87%-100%), but discrepant sensitivity (19.3%-98.9%). There are three studies that described the AUC: Zhou *et al*[41] reported that autoantibodies against seven TAAs could distinguish GC patients from healthy subjects with an AUC of 0.73. Zayakin *et al*[42] showed that 45 autoantibodies could distinguish GC patients from healthy subjects with an AUC of 0.79, while Meistere *et al*[43] reported an AUC of 0.60. These ten studies of autoantibodies in GC vary greatly regarding the number of autoantibodies measured (ranging from 2 to 102), the techniques used to detect the autoantibodies, the definition of suitable control groups, and the methods used to normalize the data and define cut-off values. Taken together, these factors may greatly hinder the clinical application of the reported biomarkers.

In general, measuring autoantibodies against TAAs has been reported to have excellent specificity but general sensitivity, which would hamper its use in clinical medicine. The biological mechanisms underlying the limitations of autoantibody sensitivity are currently unknown. Additionally, the heterogeneity of TAAs among cancer patients is very high, and one cancer-specific autoantibody usually has a low probability of detection and is thus unlikely to have statistical significance. Therefore, recently published studies are likely to be statistically inefficient. However, diagnostic biomarker panels result in the low repeatability of initial results and reduce the diagnostic value of autoantibodies, but this issue could be remedied by analyzing combinations with good statistical significance.

PERSPECTIVES

In general, the field of CTCs, ctDNAs and autoantibodies is stimulating discovery regarding the tumor recurrence and metastasis, but it is still in the early stages. The

Table 2 Detection of cell-free tumor DNA in gastric cancer

Candidate biomarkers	Sample size	Sample type	Method/technology	Diagnostic value/outcome	Ref.
Total cell-free DNA level b-actin	GC = 53, HC = 21	Plasma	qPCR	AUC = 0.75, $P < 0.0001$	Sai *et al*[31], 2007
DNA methylation markers RPRM (Reprimo)	GC = 43, HC = 31	GC tissues and plasma	MSP	95.3% GC, 9.7% HC, $P < 0.00001$; Strong correlation between methyl status in tissues and plasma	Bernal *et al*[74], 2008
Gene amplification MYC gene copy number (MYC/GAPDH ratio)	GC = 57, HC = 39	Tissues and plasma	qPCR	AUC = 0.816; Strong positive correlation between MYC levels in GC tissues and plasma ($r = 0.342$; $P = 0.009$)	Park *et al*[75], 2009
RUNX3	GC (preoperative) = 65, GC (postoperative) = 43, HC = 50	Tissues and serum	qMSP	AUC = 0.8651, Sn = 95.5%, Sp = 62.5%; Decrease after surgical resection	Sakakura *et al*[76], 2009
KCNA4 + CYP26B1	GC = 46, GPL = 46, HC = 30	Serum	Discovery: Methylation microarray in tissues; Testing: MSP	AUC = 0.917, Sn = 91.3%, Sp = 92.1%	Zheng *et al*[77], 2011
SLC19A3	Discovery: GC = 45, HC = 60; Validation: GC = 20, HC = 20	Plasma	MSRED-qPCR	Increased in GC, $P < 0.0001$	Ng *et al*[78], 2011
Alu DNA sequences	GC = 54, HC = 59	Plasma	Alu81-qPCR	AUC = 0.784, Sn = 75%, Sp = 63%	Park *et al*[32], 2012
FAM5C + MYLK	GC = 58, GPL = 46, HC = 30	Serum	Discovery: MeDIP in cell lines; Testing: MSP	AUC = 0.838, Sn = 77.6%, Sp = 90% for GC *vs* HC; Sn = 30.4% for GPL *vs* HC; Decrease after surgical resection	Chen *et al*[79], 2012
XAF1	GC = 202, HC = 88	Tumor tissues and serum	qMSP	AUC = 0.909, $P < 0.0001$; 83.9% concordance between tissues and serum	Ling *et al*[33], 2013
Total cfDNA level	Early GC = 16; advanced GC = 14; HC = 34	Plasma	Measurement of cfDNA concentration	AUC = 0.991, Sn = 96.67%, Sp = 94.11% for GC *vs* HC	Kim *et al*[80], 2014
HER2 + MYC	GC = 81; gastritis = 63; HC = 32	Plasma and tissues	FISH and qPCR	AUC = 0.850, Sn = 69%, Sp = 92%	Park *et al*[34], 2014
HER2 gene copy number (HER2/RPPH1 ratio)	Discovery: GC = 52 (pre and post-operative treatment), HC = 40;Validation: GC = 25 plasma	Plasma and tissues	qPCR	AUC = 0.746, Sn = 53.9%, Sp = 96.7%; Positive correlation between GC tissues and plasma ($r = 0.424$; $P = 0.00721$); Decrease in post-treatment plasma in HER2 + GC cases; Sn = 66.7%, Sp = 100%	Shoda *et al*[35], 2015
TP53	GC = 6	Plasma	Parallel sequencing	ctDNATP53 mutation in three out of six patients (50%)	Hamakawa *et al*[81], 2015
AKT1, AKT3, PIK3CA, PTEN, ARID1A, TP53 and BRAF	GC = 277	Plasma and tissues	MassARRAY system	32 out of 94 patients (34%) with a tissue mutation had a corresponding mutation in plasma	Fang *et al*[82], 2016
HER2	GC = 70	Plasma and tissues	dual-color ISH assay	ctDNA had a high concordance of HER2 amplification with tumor tissues(91.4%, Kappa index = 0.784, $P < 0.001$)	Gao *et al*[83], 2017
HER2	GC = 60; HC = 30	Plasma and tissues	digital droplet PCR	The preoperative plasma HER2 ratio correlated with the tumor HER2 status ($P < 0.001$); Sn = 73.3%, Sp = 93.3%	Shoda *et al*[36], 2017

AUC: Area under the curve; GC: Gastric cancer; GPL: Gastric precancerous lesions; HC: Healthy controls; MeDIP: Methylated DNA immunoprecipitation; MSP: Methylation-specific PCR; MSRED-qPCR: Methylation-sensitive restriction enzyme digestion and real-time quantitative PCR; Sn: Sensitivity; Sp: Specificity; FISH: Fluorescence in situ hybridization.

transformation of these blood biomarkers into conventional clinical indicators is hampered by the absence of consistency among different technical methods. The CellSearch system is the first standardized semi-automatic technique approved by the FDA to enrich and detect CTCs in patients with breast, prostate or colorectal cancer. Many studies have shown that the results of CTCs detection with the CellSearch system could serve as a clinical prognostic and therapeutic effectiveness indicator for these cancers. Recently, a few studies have shown that detection of CTCs in GC patients using the CellSearch system could be used for staging, predicting

Table 3 Detection of autoantibodies against tumor associated antigens in gastric cancer

Biomarker signature description	Technology	Study design	Sample size (GC/controls)	Diagnostic value	Ref.
2 TAAs-p62 and Koc	ELISA	GC *vs* HC	135/82	Sn = 19.3%, Sp = 97.6%, P < 0.01	Zhang *et al*[84], 2001
3TAAs-IQGAP3, KRT23 and REG3A	PARSE assay	GC *vs* HC (age and sex matched)	48/46	Sn = 22.9%, Sp = 100%, P < 0.001	Xu *et al*[85], 2012
3 TAAs-p16, p53 and c-myc	ELISA	GC *vs* HC	74/82	Sn = 21.6%, Sp = 97.6%; P < 0.001	Looi *et al*[86], 2006
6 TAAs-p53, Hsp70, HCC-22-5, PrxVI, KM-HN-1 and p90	ELISA	GC *vs* HC, training set	100/79	Sn = 49.0%, Sp = 92.4%, P < 0.01	Hoshion *et al*[87], 2017
		GC *vs* HC, validation set	248/74	Sn = 52.0%, Sp = 90.5%, P < 0.01	
7 TAAs - p53, C-myc, p16, IMP1, Koc, p62 and Survivin	ELISA	Cardia GC *vs* HC	88/140	AUC = 0.73, Sn = 64%, Sp = 87%, P < 0.001	Zhou *et al*[41], 2015
7 TAAs - C-myc, Cyclin B1, IMP1, Koc, P53, p62 and Survivin	ELISA, fixed cut-off	GC *vs* HC	91/346	Sn = 52.7%, Sp = 89.9%, P < 0.01	Zhang *et al*[88], 2003
	ELISA, individual cut-off	GC *vs* HC	91/346	Sn = 98.9%, Sp = 93.1%, P < 0.001	Koziol *et al*[89], 2003
45 T7 phage-displayed TAA clones (including NY-ESO-1, DDX53, MAGE antigens *etc*.)	T7 phage displayed TAA microarray	GC *vs* HC (age and sex matched)	T:100/100	AUC = 0.79, Sn = 59%, Sp = 90%, P < 0.001	Zayakin *et al*[42], 2013
			V:235/213	AUC = 0.64, Sn = 58.7%, Sp = 55%, P < 0.001	
		GC *vs* gastritis	235/100	AUC = 0.76, Sn = 58.7%, Sp = 81.5%, P < 0.001	
		GC *vs* gastric ulcer	235/54		
64 TAAs (including MAGEA4, CTAG1, TP53, ERBB2_C and SDCCAG8 antigens *etc*.)	Bead-based multiplex serology	GC *vs* HC	T:155/224	Sn = 0-12%, Sp = 98%; P > 0.05	Werner *et al*[90], 2016
		GC *vs* HC	V:146/97	Sn = 32%, Sp = 87%; P < 0.001	
102 TAAs (including CTAG1B/CTAG2, DDX53, IGF2BP2, TP53 and MAGEA3 antigens *etc*.)	A recombinant antigen microarray	GC *vs* HC	829/929	AUC = 0.60, Sn = 21%, Sp = 91%, P < 0.001	Meistere *et al*[43], 2017

AUC: Area under the curve; GC: Gastric cancer; HC: Healthy controls; ND: Not determined; Sn: Sensitivity; Sp: Specificity; TAA: Tumor associated antigen; TSA: Tumor specific antigen; T: Training; V: Validation.

patients' overall survival and evaluating the treatment effectiveness. However, large-scale clinical studies are needed to further validate the important role of CTCs and to explore an applicable cut-off value for the CTCs score in GC patients.

Although various methods and techniques have been recommended for ultimately establishing an applicable, sensitive and real-time monitoring system using circulating blood, few methods can currently be applied in clinical practice. Large-scale clinical trials and further exploration of the biology and significance of blood biomarkers might solve the associated problems and improve their application as blood biomarkers. Therefore, the exploration of revolutionary blood biomarkers, such as CTCs, ctDNAs and autoantibodies, could provide many advantages for gastric cancer patients and improve their clinical outcomes in the future.

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MINIREVIEWS

Predictive factors for anastomotic leakage after laparoscopic colorectal surgery

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Abstract

Every colorectal surgeon during his or her career is faced with anastomotic leakage (AL); one of the most dreaded complications following any type of gastrointestinal anastomosis due to increased risk of morbidity, mortality, overall impact on functional and oncologic outcome and drainage on hospital resources. In order to understand and give an overview of the AL risk factors in laparoscopic colorectal surgery, we carried out a careful review of the existing literature on this topic and found several different definitions of AL which leads us to believe that the lack of a consensual, standard definition can partly explain the considerable variations in reported rates of AL in clinical studies. Colorectal leak rates have been found to vary depending on the anatomic location of the anastomosis with reported incidence rates ranging from 0 to 20%, while the laparoscopic approach to colorectal resections has not yet been associated with a significant reduction in AL incidence. As well, numerous risk factors, though identified, lack unanimous recognition amongst

researchers. For example, the majority of papers describe the risk factors for left-sided anastomosis, the principal risk being male sex and lower anastomosis, while little data exists defining AL risk factors in a right colectomy. Also, gut microbioma is gaining an emerging role as potential risk factor for leakage.

Key words: Laparoscopic colorectal surgery; Colorectal surgery; Anastomotic leakage; Laparoscopy; Risk factor; Rectal cancer; Diverting stoma

Core tip: In colorectal surgery, knowledge and prevention of possible complications are mandatory. Anastomotic leakage is a major issue in laparoscopic colorectal surgery and furthermore, its etiology is not fully understood. The aim of this review was to evaluate the current literature to identify patient-related and perioperative risk factors for leakage in patients undergoing colorectal resection by laparoscopy. Full awareness of risk factors is essential for identifying high-risk patients and properly select them for diverting stomas in order to mitigate potential severe clinical consequences of anastomotic leakage.

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INTRODUCTION

Every colorectal surgeon during his or her career is faced with anastomotic leakage (AL); one of the most dreaded complications following any type of gastrointestinal anastomosis due to increased risk of morbidity, mortality, overall impact on functional and oncologic outcome and drainage on hospital resources[1].

Several definitions of AL can be found in the literature and therefore lack of a standardized definition can partly explain the considerable variations in AL reported rates among clinical studies[1,2]. More generally, AL is grouped together with all conditions characterized by clinical or radiologic features of anastomotic dehiscence in accordance with the United Kingdom Surgical Infection Study Group[3-5]. In order to make a valid comparison of the different existing studies characterizing AL, in 2010 specific guidelines on defining AL following rectal surgery were published by the International Study Group of Rectal Cancer. According to these guidelines AL is defined as a defect of the intestinal wall at the anastomotic site (including suture and staple lines of neorectal reservoirs) leading to a communication between the intra- and extraluminal compartments[4].

The etiology of AL is considered multifactorial. Colorectal leak rates have been found to vary according to the anatomic location of the anastomosis, with distal colorectal, coloanal and ileoanal leak rates ranging from 1% to 20%, colocolonic leak rates from 0% to 2%, and ileocolonic leak rates from 0.02% to 4%[6-9]. After almost a century of investigation, a number of patient-related and perioperative factors, as well as technical considerations, have been implicated as risk factors for AL. In some instances conclusive recommendations are firmly justified whereas others are still open to debate[1,10]. Many authors have tried to compose nomograms in order to predict the risk of AL yet, despite the significance of such scores, they are not frequently used in clinical practice[11-13].

Surgical techniques and technologies as well as perioperative care have greatly evolved over the past several decades. The laparoscopic approach is now increasingly considered the standard of care in almost all colorectal diseases due to improved short-term postoperative results with no detrimental effects on oncological outcomes when compared to open surgery[14,15]. Laparoscopy is associated with providing a better view of the surgical field, less intraoperative blood loss, reduced tissue trauma and lower inflammatory response[16]. Despite these reported advantages the laparoscopic approach for colorectal resections has not been associated with a significant reduction in AL incidence until now. Most published studies and meta-analyses reported similar rates to open surgery[17,18]. Recently a retrospective analysis of 25097 patients undergoing colectomy for colon cancer revealed that, after adjusting for other factors, patients who had undergone open or converted procedures were nearly twice as likely to suffer from AL when compared to those subject to laparoscopy. This significant difference suggests that there may be true benefits to minimally invasive colon resection as it relates to AL[19].

The aim of this review was to evaluate the current literature in order to identify patient-related and perioperative risk factors for AL in patients undergoing colorectal resection by way of the laparoscopic approach.

SEARCH STRATEGY AND QUALITY ASSESSMENT

A systematic review of literature was conducted according to the PRISMA statement[20]. A literature search was carried out in electronic databases (PubMed, MEDLINE, EMBASE) in order to retrieve all papers related to AL risk factors during laparoscopic colorectal surgery. The following search string was used: [(colorectal OR colon OR rectal OR colon surgery OR rectal surgery OR colorectal surgery) AND (anastomotic leak OR leakage OR fistula OR dehiscence) AND (risk factor OR risk) AND (laparoscopic OR laparoscopy)]. Two independent researchers analysed each article first by title and abstract, and subsequently by the full text and extracted the relevant data. In case of disagreement a

third researcher was consulted. A manual search was conducted to identify further relevant studies. All papers not in the English language, reviews, meta-analyses and study-protocols were excluded. Both randomized and non-randomized studies were included in the review. The papers were divided into the following categories according to anastomosis location: (1) Right-sided anastomosis: all anastomoses involving the ileum and the colon such as in a right colectomy; (2) left-sided anastomosis: all anastomoses involving the left colon (colocolonic, colorectal and coloanal anastomoses) or the ileum (ileorectal and ileoanal); and (3) all types of resection: both right-sided and left-sided anastomoses.

According to PRISMA guidelines, the selection flow diagram is reported in Figure 1.

The JADAD score was used to assess the quality of randomized controlled trials (RCTs) and papers with a score of $\geqslant$ 3 were included in the analysis. The methodological quality of non-randomized surgical studies was assessed with a MINORS score. A score $\geqslant$ 10 for non-comparative studies and $\geqslant$ 14 for comparative studies was fixed as a threshold for inclusion in the analysis[21,22].

RISK FACTORS FOR LEAKAGE

Right-sided anastomosis

After the literature review and quality assessment, one RCT and nine non-randomized papers were included in the analysis. Kwak *et al*[23] reported their retrospective series of 423 patients who had undergone laparoscopic colonic resection and anastomosis for appendix or right colon cancer. The overall leakage rate over the 8-year study period was 3.78% (16/423 patients). Among patient-related factors, habitual smoking was found to be significantly associated (P = 0.007) with AL with an odds ratio (OR) of 6.529 and it was suggested that vascular ischemia from nicotine-induced vasoconstriction and microthromboses, together with carbon monoxide-induced cellular hypoxia, inhibit anastomotic circulation in smokers[24]. Neoadjuvant chemotherapy correlated with AL (6.3% in the leakage group compared to the 0.5% in the non-leakage group, P = 0.007) however the sample size of only 3 patients was too small to be clinically relevant[23]. Among operative factors, longer operating time was found to be significantly associated with leakage (OR = 1.024, P < 0.001).

Intracorporeal anastomosis

Laparoscopic right colectomy with intracorporeal anastomosis (IA) is reported to have some benefits in terms of enhanced postoperative recovery in comparison with laparoscopic-assisted right colectomy with extracorporeal anastomosis (EA)[25]. Both approaches appear to achieve similar results in terms of AL occurrence. Definitive conclusions are difficult to draw, however due to the nature of the published studies and the heterogeneity of surgical techniques used in fashioning the EA, including both manual, totally-stapled, and stapled-manual[26]. Vignali *et al*[26] published an interim analysis of the first RCT analyzing the role of intracorporeal stapled versus extracorporeal stapled anastomosis following laparoscopic right colectomy using a standardized approach. In their series of 60 patients (30 EA *vs* 30 IA) no significant difference was observed between the two groups with respect to AL (6.6% in the IA group versus 0% in the EA group, P = 0.39). In the largest multicenter study comparing IA and EA for 512 right-sided colorectal cancers, the incidence of leak or dehiscence was 4.19% (12 patients) in the IA group and 5.50% (12 patients) in the EA group (P = 0.53)[3]. Similarly, in a case-matched study, Vignali *et al*[27] compared the outcomes of IA (64 patients) versus EA (64 patients) in an obese population [body mass index (BMI) > 30 kg/m^2]. Clinically evident anastomotic leaks occurred in 4.7% of the patients in the IA group *vs* 7.8% in the EA group (P = 0.71). Also, in a retrospective multicentric comparative study including 195 patients, multivariate analysis revealed a trend towards lower risk of clinical AL (requiring percutaneous or operative intervention) with IA that failed to reach statistical significance (adjusted OR = 0.29, P > 0.05)[28]. Other retrospective series found no significant differences in incidence of anastomotic leaks between the two techniques[29-32] . With regards to IA, a single-centre retrospective series of 162 patients found that double-layer closure of enterotomy was associated with a significantly lower incidence of AL compared to single-layer closure (1.2% in DL *vs* 7.8% in SL, P = 0.044) after mechanical ileocolic anastomosis[33].

Left-sided anastomosis

Following a literature review and quality assessment, 5 RCTs and 34 non-randomized studies were included in the analysis (Table 1).

Patient-related factors

Male sex: AL was reported to be more common amongst men which may be reflective of the fact that technical difficulties can be intensified in male patients due to their narrow pelvises[34]. In a retrospective study of 296 patients who had undergone laparoscopic anterior resection (LAR), male gender was a significant risk factor with an OR of 18.0 at multivariate analysis[35]. Similarly, Kim *et al*[36] analyzed risk factors for AL in 312 LARs for both extraperitoneal and intraperitoneal disease location. Male gender was the only risk factor identified and leakage was 13.2 times higher in men than in women. Tanaka *et al*[37] 's prospective trial also found that men are at a higher risk for leakage (OR = 4.12). In a multicenter analysis of 1609 patients with rectal cancer, male gender was a significant risk factor amongst all patients [hazard ratio (HR) = 1.943] and particularly amongst patients without defunctioning stoma (HR = 3.468)[34].

BMI: Two papers have shown that BMI could also be a risk factor for AL. In a series of 1059 patients undergoing laparoscopic sigmoidectomy for diverticulitis, BMI $\geqslant$ 35 kg/m^2 was independently associated (OR = 2.3) with AL

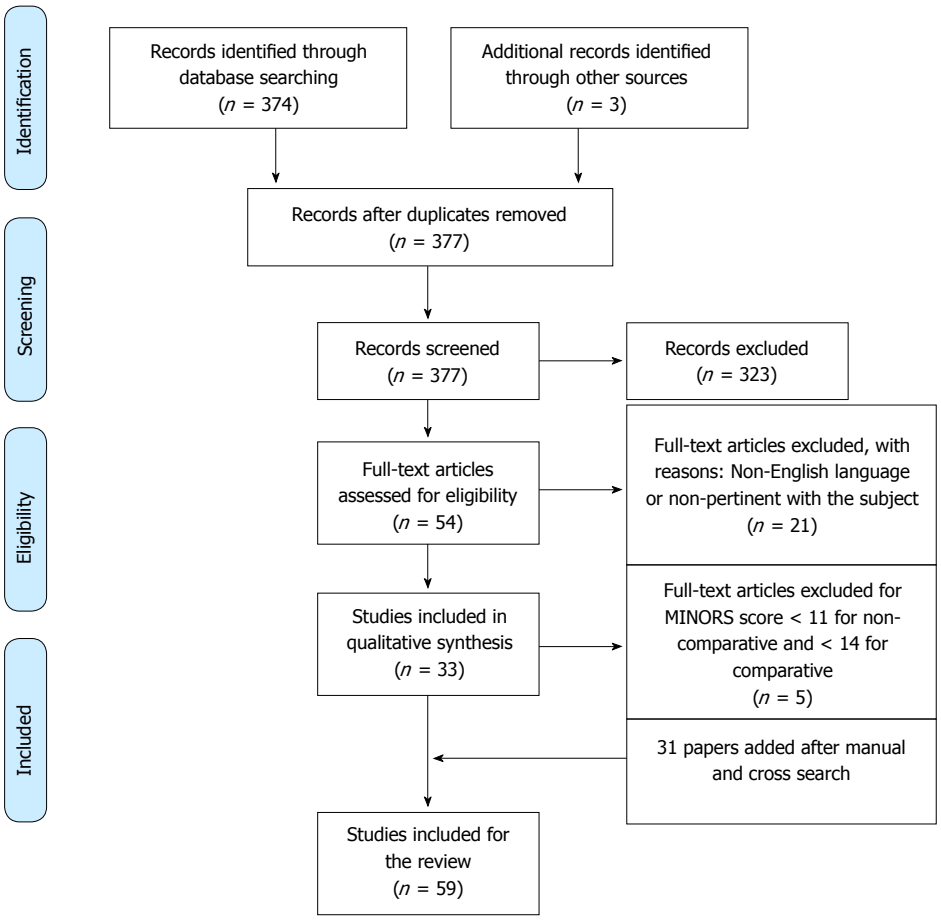

Figure 1 Selection flow diagram according to PRISMA guidelines.

and/or postoperative abscess both in an intent-to-treat analysis and amongst laparoscopically completed cases[38]. Yamamoto *et al*[39] found that BMI was independently predictive for developing AL (OR = 1.479).

Preoperative nutritional status: Malnutrition impairs anastomotic healing by affecting collagen synthesis or fibroblast proliferation. Impaired preoperative nutritious status defined as anemia or hypoproteinemia (hemoglobin $\leqslant$ 100 g/L or albumin $\leqslant$ 32 g/L) was found to be significant (P = 0.047) at a univariate analysis in a retrospective series of 132 patients undergoing LAR for cancer[40]. This finding was not confirmed at multivariate analysis (P = 0.253).

Neoadjuvant therapy: Park *et al*[34] reported that preoperative chemoradiation was a risk factor for leakage in their subgroup analysis of patients without defunctioning stoma (HR = 2.418), but not in their analysis of all patients after LAR for cancer. Hamabe *et al*[35] reported an association between AL and neoadjuvant chemotherapy with an OR of 3.5 at multivariate analysis.

Tumor size and stage: Tumor size may represent one of the risk factors for AL following LAR. This procedure involves surgery in an anatomically narrow space and as tumor size and/or stage increases, intrapelvic manipulation becomes restricted and rectal transection more challenging[41]. Moreover, patients with a tumor larger in size or more advanced in TNM staging usually suffer from a worsened systemic physical status[40]. In a series of 154 rectal cancer patients, tumor size $\geqslant$ 5 cm in diameter was associated with a 4-fold higher risk of leakage[42]. Zhu *et al*[40] found that tumors larger than 3 cm in diameter, as well as TNM stage, were independently associated with leakage.

Post-operative hypoalbuminemia: Post-operative nutritional status monitoring could be a good way to identify patients with high risk of post-operative AL. In a retrospective series of 200 patients undergoing laparoscopic curative surgery for colorectal cancer, the average serum albumin levels on POD1 and POD3 were significantly lower in the AL group compared to the non-leakage group (P < 0.0005)[43].

Post-operative diarrhea: Ito *et al*[44] reported an association between postoperative diarrhea and occurrence of AL, with an OR of 86.3. The authors speculated that early postoperative diarrhea increases endoluminal pressure at the anastomotic site. Furthermore, leaking of watery stool through the anastomosis may lead to the

Table 1 Studies involving laparoscopic colorectal procedures with left-sided anastomosis

Author	Year	No. of patients	Overall leak rate (*n*)	Risk factor identified
Ito *et al*[8]	2008	180	5.0% (9)	TME N° of staplers firing (≥ 3)
Kim *et al*[36]	2008	266	6.4% (17)	Male sex
Pugliese *et al*[66]	2008	157	10.8% (17)	Conversion
Kim *et al*[47]	2009	270	6.3% (17)	Tumor location in middle or lower rectum
Zhu *et al*[40]	2010	132	9.1% (12)	Tumor size (diameter ≥ 3 cm) Distance from the anal verge (≤ 6 cm) TNM stage
Choi *et al*[45]	2010	156	10.3% (16)	Anastomotic level ≤ 5 cm from the anal verge Long operation time (≥ 270 min)
Huh *et al*[46]	2010	223	8.5% (19)	Extraperitoneal location of tumor Operative time > 220 min
Kayano *et al*[41]	2011	250	10.0% (25)	Male sex Multiple stapler firings (≥ 2)
Akiyoshi *et al*[49]	2011	363	3.6% (13)	Middle/low rectal cancer Lack of pelvic drain
Yamamoto *et al*[39]	2012	111	5.4 (6)	BMI
Hinoi *et al*[68]	2013	888	9.3% (83)	LCA ligation in LAR
Park *et al*[34]	2013	1609	6.3% (101)	Male sex Low anastomosis (< 7 cm) Preoperative chemoradiation Advanced tumor stage Perioperative bleeding (≥ 2 transfusions) Multiple firings of the linear stapler (> 3)
Kawada *et al*[42]	2014	154	12.3% (19)	Tumor size > 5 cm Operative time > 300 min Intraoperative bleeding > 100 mL Stapler firings > 3 Precompression before stapler firing
Majbar *et al*[65]	2016	131	16.0% (21)	Conversion to open surgery
Silva-Velazco *et al*[38]	2016	1059	9% (95)	BMI ≥ 35 kg/m^2 N° of staplers firing Longer operative time
Van Praagh *et al*[74]	2016	16	50% (8)	Low diversity of gut microbiota High presence of Lachnospiraceae
Hamabe *et al*[35]	2017	296	8.1% (24)	Male sex Distance from anal verge < 7 cm Neoadjuvant chemotherapy
Lee *et al*[48]	2017	128	0.78% (1)	Stapler firings > 2 Distance from anal verge
Tanaka *et al*[37]	2017	395	8.4% (33)	Male sex Absence of transanal tube
Ito *et al*[44]	2017	69	15.9% (11)	Absence of transanal tube Post-operative diarrhea
Shimura *et al*[43]	2018	196	5.61% (11)	Post-operative hypoalbuminemia
Van Praagh *et al*[75]	2018	123	23.6% (29)	Bacteroidaceae Low diversity of gut microbiota High presence of Lachnospiraceae Anostomosis covered with C-Seal

BMI: Body mass index; LCA: Left colic artery; LAR: Laparoscopic anterior resection; TME: Total mesorectal excision.

development of localized or generalized pelvic infection.

Operative factors

Level of anastomosis: The distance of the anastomosis from the anal verge is regarded as the most important predictive factor for leakage. Several studies have shown that the lower the anastomosis, the higher the risk of leakage[34,40,45-47]. Hamabe *et al*[35] reported that the leak rate was 3.4 times higher for tumors located less than 7 cm from the anal verge. An anastomotic level within 5 cm from the anal verge was a risk factor for leakage at both univariate (P < 0.001) and multivariate analysis (OR = 6.855; 95%CI: 1.271-36.964; P = 0.025) in a series of 156 patients undergoing LAR without diverting ileostomy[45]. In this study the AL rate was 10 times higher (20.6% *vs* 2.3%) when the anastomotic region was located within 5 cm of the anal verge. Accordingly, low levels of anastomosis accompanied with total mesorectal excision (TME) were independently associated with leakage[8]. In their series of 128 patients, Lee *et al*[48] reported that low distance from the anal verge could be a risk factor for leakage but that, due to their very low leak rate, they could not demonstrate it.

Number of linear stapler firings: A disadvantage in laparoscopic surgery is that rectal transection may be more difficult than in open surgery[41]. The narrow space in which to insert the stapler, inadequate traction and a suboptimal cutting angle may necessitate multiple applications of the linear stapler[34]. The concern about number and direction of stapler firings has been reported by many surgeons. In a series of 180 cancer patients, three or more stapler firings during rectal division significantly increased the risk of AL after the laparoscopic double stapling technique (OR = 4.6)[8]. Rectal division through the right-lower port required more stapler firings than division through the suprapubic port, especially in the TME group, and a smaller percentage of patients required three or more staples for vertical rectal division than for transverse division (15% *vs* 45%, *P* = 0.03). Park *et al*[34] also reported that a number of linear stapler firings > 3 was a risk factor for leakage (HR = 7.849). Choi *et al*[45] found that 16.7% of the cases in which 3 or more linear staplers were used had AL, whereas only 6.8% of the cases in which 2 or fewer linear staplers were used had leakage. Though there was no statistical significance to this difference (*P* = 0.068), the authors claimed that efforts to reduce the number of linear staplers to 2 or less seemed to be warranted. Kim *et al*[47] found that more than 2 stapler firings were associated with leakage at univariate analysis. The number of stapler firings increased significantly in men (*P* = 0.023), in patients with a tumor at a lower level (*P* = 0.034), and in those with longer operating times (*P* < 0.001). Several other authors reported an association between multiple linear stapler firings and AL incidence[38,41,42]. In Lee *et al*[48]'s series, this association could not be statistically proven due to the very low leak rate.

Diverting stoma: Although evidence regarding the clinical benefit of fecal diversion is conflicting, it is generally agreed that creation of a diverting stoma (DS) can reduce the clinically adverse effects of AL, including fecal peritonitis and septicemia, rather than preventing leakage. In a retrospective series of 69 patients undergoing LAR[44], no significant difference between DS group and no-DS group in terms of AL incidence (15.4% *vs* 16.3%) was noted. Although AL was observed in four patients in the DS group, none of them developed AL grade C. In contrast, 57.1% (4/7 cases) of the patients in the no-DS group developed AL grade C, but this difference did not reach statistical significance[44].

In the series from Park *et al*[34] (1609 patients) defunctioning stoma did not significantly reduce risk of AL (OR = 0.649, *P* = 0.154 at multivariate analysis). Similarly, in a series of 363 LARs, the incidence of AL was 4.8% in patients with covering stoma versus 3.3% in patients without stoma (*P* = 0.4718)[49]. Other studies reported similar findings[38,41,42].

In a series of 296 low LARs for cancer[35], AL was observed in 5.5% of patients with DS and in 8.7% of patients without DS (OR = 0.60, *P* = 0.4243 at univariate analysis). Based on the two risk factors (sex and anal verge distance) patients were stratified according to risk for AL occurrence. The incidence of AL was 8.1% in the overall population compared to 23% in high-risk patients (males with tumors less or equal than 7 cm from the anal verge). Within this group, DS creation significantly reduced the AL rate (*P* = 0.0363) as the rate of AL occurrence was 10.7% in patients for whom a DS was created compared to 33.3% in patients without a DS. The occurrence of AL in the low-risk group was not influenced by DS creation (*P* = 0.2443). Based on the findings of this study, DS may help prevent the occurrence of AL in a high-risk population.

Transanal TME: Transanal TME (TaTME) represents the latest advanced surgical access technique for pelvic dissection and anastomosis during rectal resection and is being implemented in clinical practice in order to overcome the technical drawbacks and limitations of standard laparoscopic TME[50] . For instance, the distal rectal transection does not involve multiple stapler firings and therefore eliminates this potential risk factor for leakage. Recently, Penna *et al*[50] analyzed 1594 TaTME cases with an anastomosis recorded on the international TaTME registry[51]. The overall anastomotic failure rate was 15.7%. This included early (within 30-d; 7.8%) and delayed (after 30 d; 2.0%) leak, pelvic abscess (4.7%), anastomotic fistula (0.8%), chronic sinus (0.9%), and anastomotic stricture in 3.6% of cases. Of 250 patients diagnosed with anastomotic failure, 219 had a defunctioning stoma created at the index operation. The reported early leak rate of 7.8% was higher than the previously published rate of 5.4% in the initial 720 registry cases[52]. The authors suggested that this value could be explained by an increased complexity of cases performed transanally, wider adoption of TaTME by surgeons at the start of their learning curve, or improved recording and reporting of adverse events on the registry. Nonetheless, the leak rate was comparable to previously reported incidences in colorectal surgery. Upon multivariate analysis, male sex, obesity, smoking, diabetes, larger tumors (> 25 mm maximum diameter), tumor height > 4 cm from anorectal junction on magnetic resonance imaging, and intraoperative blood loss of ⩾ 500 mL were risk factors for early AL. These factors are similar to those identified in previous studies on laparoscopic rectal resections. Significantly more cases that did not have a defunctioning stoma developed early symptomatic AL compared with those that were defunctioned (12.4% *vs* 7.2%, OR = 0.547, *P* = 0.015). However, the presence of a defunctioning stoma did not appear to significantly influence incidence of anastomotic failure in this cohort. Anastomotic technique (manual versus stapled) was not identified as a risk factor for early AL, although the manual technique significantly increased the risk of late stricturing. A few published studies have compared laparoscopic and transanal TME with respect to AL rates. A RCT including 100 patients found a leak rate

of 2% in the transanal group compared to 10% in the laparoscopic group, without a significant difference (P = 0.204)[53]. Other retrospective matched case-control trials did not find any statistically significant difference in terms of AL rates between the two approaches[54-57]. Results from the recently commenced RCTs comparing TaTME with laparoscopic TME may provide some robust data in the future[58,59].

Circular stapler: In animal models pre-compression before firing with a circular stapler was demonstrated to reduce intestinal wall thickness and acquire optimal anastomosis[42]. Only one published study reported that long pre-compression time before firing was associated with AL at a multivariate analysis (OR = 4.85)[42]. The diameter of the circular stapler was not found to be a risk factor for leakage in three studies[34,45,46].

Intraoperative endoscopy: The usual ways of assessing the integrity of colorectal anastomosis such as the air leak test, direct laparoscopic visualization and inspection of doughnuts may be suboptimal methods for predicting anastomotic complications. The use of intraoperative endoscopy (IOE) allows direct visualization and testing with the air leak test for anastomotic defect or bleeding, inadvertent bowel wall injury at the anastomotic site, adequacy of distal margins, vascularity of the anastomosis, and unsuspected distal lesions or stricture at the preoperative assessment[60]. Li *et al*[60]compared 107 patients who had undergone routine IOE to 137 patients who had undergone selective IOE during laparoscopic colorectal surgery. A 5.7-fold increase in anastomotic complications was observed in the selective IOE group although the difference was not statistically significant due to their small sample size. AL incidence was comparable between the two groups.

Indocyanine green fluorescence angiography: Intraoperative assessment of perfusion at the site of anastomosis with indocyanine green (ICG) has been increasingly considered a potential intraoperative tool that could be used to ensure adequate perfusion, possibly leading to a reduction in the AL rate. Most published studies focused on the change of surgical strategy (site of resection and/or anastomosis) due to the subjective recording of hypoperfusion after ICG fluorescence angiography (FA). However, its capacity to reduce AL incidence needs to be confirmed in large RCTs. Boni *et al*[61] compared 42 patients undergoing LAR with ICG angiography to a historical control group of 38 patients operated on without the use of angiography. No clinically relevant leaks were observed in the FA group, whereas two leaks were reported in the case-matched group. This difference is not likely to be statistically significant due to the limited number of patients analyzed. Jafari *et al*[62] published a prospective multicenter clinical trial including 139 patients who had undergone laparoscopic left-sided colectomy and anterior resection. The overall AL rate was 1.4%. FA changed surgical plans in 11 (7.9%) patients, with the majority of changes occurring at the time of transection of the proximal margin (7%). No AL was recorded amongst this subgroup of patients. In a prospective single-institution study of 68 patients undergoing laparoscopic resection for left-sided colorectal cancers, AL occurred in 16.7% of the poor perfusion group based on ICG fluorescence imaging, whereas none of the patients in the good perfusion group had AL. When further focusing on LAR, the AL rate was 10.7%. Leak occurred in 30% of the poor perfusion group, whereas no leak took place in the good perfusion group[63].

Fibrin glue: Fibrin glue application over the stapled anastomosis was not found to be significantly associated with leakage following laparoscopic rectal cancer surgery without stool diversion[46].

Operative time: Prolonged operations may reflect intraoperative difficulties especially in critical patients. Therefore operative time was investigated as a possible risk factor for AL. Silva Velazco *et al*[38] found an increasing OR of 1.03 for every 30 minutes of surgical duration. Several other authors have shown that prolonged operative time can be associated with leakage, with a reported threshold varying from 220 to 300 minutes[42,45,46].

Conversion: Conversion was found to be a controversial topic in the literature, with some authors reporting higher morbidity and mortality in converted patients, while others reporting outcomes comparable to laparoscopy. In a single-institution retrospective analysis of 1114 patients undergoing elective laparoscopic resection for non-metastatic colorectal cancer, the conversion rate was 10.9%. The most common reason for conversion was a locally advanced tumor followed by obesity and adhesions. Conversion was associated with significantly longer operative time and greater blood loss. No statistically significant differences in terms of an overall 30-day postoperative morbidity rate were observed between the converted and laparoscopic cases (16.4% *vs* 15.7%; P = 0.849) regardless of tumor location (colon *vs* rectum). In particular, no statistically significant differences were observed between the groups in terms of the AL rate (3.3% *vs* 4.9%; P = 0.416)[64].

In contrast, Majbar *et al*[65] in their retrospective study reported an association between conversion and AL at multivariate analysis (OR = 2.86). Similarly, in a series of 157 patients undergoing LAR for adenocarcinoma, Pugliese *et al*[66] reported a leak rate of 41% in converted patients compared to 8% in non-converted patients, with a 7.9-fold higher risk for developing a leak in the latter group.

Left colic artery ligation: The level of vascular ligation may affect blood supply to the anastomosis and subsequently anastomotic healing. Left colic artery (LCA) preservation results in increased blood supply for

anastomosis after anterior resection, even in cases of the 5% of patients lacking a marginal artery in the left colic flexure resulting in ischemia on the proximal side of anastomosis[67]. The decision to perform a high or low tie of the inferior mesenteric artery during laparoscopic left-sided colorectal resections is controversial. In a multicenter retrospective study by 20 institutions in Japan, Hinoi *et al*[68] found that LCA preservation is a significant factor for low leakage rates after LAR for middle and low rectal cancers, regardless of tumor size, extent of lymph node metastasis, and extent of excision. In their series of 888 patients the overall incidence of anastomotic leak was 9.3%. LCA preservation was associated with a leak rate of 7.4% compared to 13.2% in the non-preservation group (P = 0.005 and < 0.001 by univariate and multivariate analysis, respectively) although this result might be biased due to the different surgical and pathological backgrounds between the two groups with more advanced cancer/stage in the LCA non-preservation group. Thus a subgroup analysis was performed on 411 patients undergoing *en bloc* radical lymph node excision associated with LCA ligation or preservation. The AL rate was 7.1% in the LCA preservation group compared to 14.5% in the LCA non-preservation group, the difference being statistically significant (P = 0.024 and 0.005, univariate and multivariate analysis, respectively). In contrast, the level of inferior mesenteric artery ligation was not found to be a risk factor for leakage in a series of 156 patients undergoing LAR without DS[45].

Pelvic drainage: Routine prophylactic drainage after colorectal anastomoses is debatable and the evidence to support its use is low[69]. A recent RCT analyzed 469 patients who underwent rectal resection with infraperitoneal anastomosis, of whom 93.6% were operated on by laparoscopy. There was no significant difference in terms of pelvic sepsis between drained and non-drained patients, either during hospital stay or at 30 days after surgery (16.1% *vs* 18.0%, P = 0.58). Early (< 5 d) versus late (> 5 d) pelvic drain removal did not affect significantly the risk of pelvic sepsis (11.6% *vs* 18.6%, P = 0.122)[70].Two retrospective studies found pelvic drainage associated with lower rates of AL after LAR, though without reaching statistical significance. Kawada *et al*[42] reported AL in 10.8% of drained patients versus 20.8% of non-drained patients (P = 0.18) in a series of 154 low LARs without DS. Similarly, in a series of 363 LARs, 2.6% of drained patients had clinical AL compared to 6.3% of non-drained patients (P = 0.11). Nonetheless lack of pelvic drain was found to be independently predictive (P = 0.0225, OR = 3.814) of leakage at a multivariate analysis[49]. Pelvic drain may prevent hematomas or seromas that constitute a fertile medium for bacteria and may promote infection which can involve the anastomosis thereby causing dehiscence. Moreover, pelvic drain may help control leaks if they do take place, leading to a less severe clinical course[71].

Trans-anal drainage: A trans-anal drainage tube was speculated by many authors to be a good way to prevent post-operative AL[37,44]. In a case series of 69 LARs, Ito *et al*[44] found that the use of trans-anal drainage is associated with lower incidence of post-operative AL. In particular, the authors explained that the presence of a trans-anal drain could prevent the unfavorable effect of post-operative diarrhea. Tanaka *et al*[37] also sustained that the absence of a trans-anal drainage tube after laparoscopic low anterior resection for stage 0/1 cancer is associated with a higher risk of post-operative AL with an OR of 3.11 at multivariate analysis. Contrarily, insertion of trans-anal drainage was reported as not correlating with AL by Hamabe *et al*[35], in high-risk patients as well.

Gut microbiota: Intestinal flora near the anastomotic site has been proposed to interact with intestinal tissue and likely affects intestinal healing[10]. Some experimental studies suggest that cues released by surgically injured tissues can lead to phenotype transformation of intraluminal microbes, turning them into pathogens. These may play a causative role in the development of AL by increased collagenase production and activation of host metalloproteinase-9[72]. Nonetheless, extensive clinical evidence on the impact of gut microbiota on postoperative anastomotic complications is lacking[73]. A pilot study compared the intestinal microbiota of 8 patients who had developed AL with 8 matched patients with healed circular stapled colorectal anastomoses without any clinical signs of AL[74]. The abundance of the *Lachnospiraceae* family was found to be significantly higher in patients who had developed AL when compared to patients who had not (P = 0.001), while microbial diversity levels were higher in the latter group (P = 0.037). Also, BMI was positively associated with the abundance of the *Lachnospiraceae* family (P = 0.022). The same study group further investigated the role of gut microbiota in the development of AL in a series of 123 "donuts" of patients where a stapled colorectal anastomosis was made[75]. In 63 patients this anastomosis was covered with a C-seal; a bioresorbable sheath stapled to the anastomosis. In the group of non-C-seal samples a high abundance of *Lachnospiraceae* and *Bacteroidaceae* and lower microbial diversity were confirmed to be strongly associated with AL. A bacterial composition that consisted of 60% or more of these two families seemed to be predictive for AL. On the contrary, other species such as *Prevotella copri* and the *Streptococcus* genus were both negatively associated with AL. The authors speculated that a disturbed microbial composition which is more easily associated with low microbial diversity[10] due to preoperative or surgical processes, may affect the metabolic balance and lack colonization resistance to pathogenic bacteria that could play a role in the development of AL. In C-seal patients where AL rates were slightly higher, it seemed that any potential protective benefits or harmful consequences of the gut microbiota composition were

Table 2 Studies involving both right and left-sided anastomoses

Author	Year	No. of patients	Overall leak rate (*n*)	Risk factor identified
Kockerling *et al*[82]	1999	894	4.2% (38)	Rectal resection Malignant disease Anastomotic level < 10 cm from the anal verge
Senagore *et al*[80]	2003	260	2.7% (7)	BMI ⩾ 30 kg/m^2
Kirchhoff *et al*[83]	2008	1316	27.7% (59)	BMI ⩾ 30 kg/m^2 Male gender Malignant neoplasia
Akiyoshi *et al*[79]	2011	1194	1.0% (12)	BMI ⩾ 30 kg/m^2 Rectal tumor location
Ris *et al*[90]	2018	504	2.4% (12)	No use of indocyanine green

BMI: Body mass index.

negated, as progression to AL was independent of the dominant bacterial composition before surgery. These observations suggested that the C-seal influences the microbial composition after introduction and that this may ultimately impair anastomotic healing.

Perioperative events: Bleeding during surgery may predispose to leakage due to hemodynamic alterations at the anastomotic site. Kawada *et al*[42] found that intraoperative bleeding at more than 100 mL was associated with significantly increased incidence of leakage (P = 0.037). Perioperative bleeding requiring 2 or more units was reported to be a risk factor for leakage in patients undergoing LAR for cancer (HR = 8.462) including those without defunctioning stoma (HR = 10.705)[34]. Also, unexpected events related to anastomosis during surgery such as instrument failure, ischemia of the proximal colon, tumor perforation and additional surgery caused by anastomotic bleeding have been significantly associated with leakage[45].

Surgeon's experience and hospital size: Two important factors that may impact the risk of AL after laparoscopic colorectal surgery are the experience of the surgeon performing the procedure and hospital volume. Two published papers report on the risk of AL as related to the experience of the surgeon and only one related to hospital size[76-78]. The individual surgeon performing the procedure, as well as hospital volume, were found to be risk factors for AL although these studies were excluded from the review after quality assessment.

Kayano *et al*[41] analyzed the AL rate of LAR during the learning curve period in a series of 250 cases that were evaluated in five groups of 50 patients each. The postoperative complication rate decreased significantly by group 5 (201-250 cases) and it was noted that AL decreased with an increase in cases although no significant difference was observed over the course of the learning curve period. Park *et al*[34] found no correlation between the incidence of AL and both hospital caseload and surgeon's TME experience.

All types of laparoscopic colorectal resections

After the literature review and quality assessment, three RCTs and seven non-randomized studies were included in the analysis (Table 2).

BMI: In a cohort of 1194 patients who had undergone laparoscopic resection for colorectal cancer, the rate of AL was significantly higher in the obese II Group (BMI > 30 kg/m^2) than in the nonobese (< 24.9 kg/m^2) and obese I (BMI 25 to 29.9 kg/m^2) groups (8% *vs* 1% and 0.4%; P = 0.0004 and 0.0002, respectively). BMI > 30 kg/m^2 was found to be independently predictive of the development of leakage (OR = 10.27)[79]. Similarly, in a series of 260 laparoscopic colectomies, the AL rate was significantly higher amongst obese (5.1%) versus non-obese (1.2%) patients[80]. On the contrary, a retrospective study on 213 patients undergoing laparoscopy colorectal surgery for inflammatory bowel disease failed to demonstrate any difference in AL rates between normal-weight patients and overweight or obese patients[81].

Tumor location: Akiyoshi *et al*[79] reported that tumor location in the rectum, rather in the colon, was found to be independently predictive of the development of AL (OR = 18.20) upon multivariate analysis. At univariate analysis, the type of operative procedure (LAR/intersphincteric resection versus others) was associated with leakage (P = 0.0004) in addition to tumor location. This finding was confirmed by a prospective multicenter study which reported on 1134 patients of whom 894 had an anastomosis[82]. In this series the leak rate was highest after LAR (12.7%) followed by left hemicolectomy (7.1%), right hemicolectomy (4%), sigmoidectomy (2.9%), and rectopexy with resection (1.25%; P = 0.0001). Surgery for benign disease was associated with a lower rate of AL (2.6%) than surgery for malignant disease (6.7%). Cancer was significantly associated with AL in a series of 1316 elective laparoscopic colorectal procedures as well[83].

Preoperative Infliximab therapy: In a retrospective series of patients undergoing elective laparoscopic resection for inflammatory bowel disease, 142 had preoperative therapy within 12 wk before surgery and were compared to 376 who had not received Infliximab.

The rate of anastomotic leaks (2.1% *vs* 1.3%, *P* = 0.81) was similar. Subgroup analysis confirmed similar rates of leakage regardless of whether patients had ulcerative colitis or Crohn's disease. According to this study, Infliximab treatment in patients refractory to conventional pharmacological therapy did not seem to affect short-term outcomes in those patients eventually submitted to surgical treatment[84].

Oral antibiotics: Recent studies[85,86] suggest that use of oral antibiotics in preoperative bowel preparation could lower infectious complications and also incidence of AL after colorectal surgery. This finding further supports a role of the gut microbiota in anastomotic integrity[67]. However data on the impact of this measure in patients specifically undergoing minimally invasive colorectal surgery are still limited[86]. In a retrospective ACS-NSQIP database analysis, in which 5291 (62.5%) patients underwent minimally invasive surgery, oral antibiotic preparation was associated with lower rates of surgical site infection (SSI) and AL for both minimally invasive and open cohorts[87]. A recent RCT by Hata *et al*[88] revealed that patients undergoing laparoscopic colorectal procedures for cancer had a lower incidence of overall SSIs (7.3% *vs* 12.8%, OR = 0.536, *P* = 0.028) when receiving oral antibiotic prophylaxis in addition to mechanical bowel preparation. However, incidence of organ/space infection was comparable to that of patients receiving mechanical bowel preparation and IV prophylaxis where 6/290 (2.1%) leaks took place in the IV group compared to 5/289 (1.7%) in the oral-IV group. In another single-center RCT including 515 colorectal cancer patients undergoing elective laparoscopic resection, IV perioperative antimicrobial prophylaxis alone was not inferior to combined pre-operative oral and IV perioperative prophylaxis with regards to SSI. AL was observed in 2.5% of the IV-only group and in 1.2% of the oral-IV group (OR = 2.01, *P* = 0.504). The authors speculated that the study was evidently underpowered to provide any conclusions regarding the contribution of oral microbial prophylaxis in reducing AL[89].

Indocyanine green fluorescence angiography: Ris *et al*[90] recently conducted a prospective phase II study of 504 patients undergoing elective bowel resection of which 85.3% were operated on by laparoscopy. The overall leak rate for colorectal operations not involving ICG fluorescence was 5.8%, compared with 2.6% with the use of ICG imaging (*P* = 0.009). Statistical significance was confirmed for left-sided resections (6.9% *vs* 2.6%, *P* = 0.005) and for low anterior resections alone (10.7% *vs* 3%), but not for right-sided operations (2.6% *vs* 2.8%, *P* = 0.928).

LIMITATIONS

Some limitations of this study have to be addressed. The major limitation lies in the retrospective nature and consequent lack of randomization of the included studies, that may lead to patient and surgeon selection bias. Second, different definitions of AL were used across the studies, which is a general problem in the literature dealing with this postoperative complication. Moreover, some series are heterogeneous in terms of type of patients, study era, surgical technique, and perioperative practice. The variable presence of DS across studies dealing with rectal resections should also be considered. Finally, some studies have relatively small sample size.

CONCLUSION

Anastomotic leakage remains a major issue in laparoscopic colorectal surgery. Current evidence about the risk factors for leaking mainly comes from non-randomized retrospective studies, most of which deal with rectal resections. In such studies, the presence of a diverting stoma should be taken into account when analysing the association between leakage and predictive factors. Several clinical variables and surgical issues have been extensively investigated, although some of them remain controversial, and it remains difficult to accurately predict the development of leakage. This suggests that the etiology of this fearsome complication is not fully understood and dictates the need for further investigations. Full awareness of risk factors is essential for identifying high-risk patients and properly select them for diverting stomas in order to mitigate the severe clinical consequences of anastomotic leakage.

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MINIREVIEWS

Hepatitis B virus pathogenesis: Fresh insights into hepatitis B virus RNA

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Abstract

Hepatitis B virus (HBV) is still a worldwide health concern. While divergent factors are involved in its pathogenesis, it is now clear that HBV RNAs, principally templates for viral proteins and viral DNAs, have diverse biological functions involved in HBV pathogenesis. These functions include viral replication, hepatic fibrosis and hepatocarcinogenesis. Depending on the sequence similarities, HBV RNAs may act as sponges for host miRNAs and may deregulate miRNA functions, possibly leading to pathological consequences. Some parts of the HBV RNA molecule may function as viral-derived miRNA, which regulates viral replication. HBV DNA can integrate into the host genomic DNA and produce novel viral-host fusion RNA, which may have pathological functions. To date, elimination of HBV-derived covalently closed circular DNA has not been achieved. However, RNA transcription silencing may be an alternative practical approach to treat HBV-induced pathogenesis. A full understanding of HBV RNA transcription and the biological functions of HBV RNA may open a new avenue for the development of novel HBV therapeutics.

Key words: Hepatitis B virus; Hepatitis B virus RNA; MicroRNA; Smc5/6; Viral replication; Hepatic fibrosis; Genome integration; Hepatocellular carcinoma

Core tip: Recently, it has been shown that hepatitis B virus (HBV) RNAs have diverse biological functions in the pathogenesis of HBV. HBV RNAs may work as sponges for host miRNAs and deregulate miRNA functions. Novel viral-host fusion RNA may be produced from HBV-DNA integration sites, which may also have pathological functions. Understanding HBV RNA transcription and the biological functions of HBV-related RNAs may open a new avenue for the development of novel HBV therapeutics that target HBV RNAs.

Sekiba K, Otsuka M, Ohno M, Yamagami M, Kishikawa T, Suzuki T, Ishibashi R, Seimiya T, Tanaka E, Koike K. Hepatitis B virus pathogenesis: Fresh insights into hepatitis B virus RNA. *World J Gastroenterol* 2018; 24(21): 2261-2268 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i21/2261.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i21.2261

INTRODUCTION

Hepatitis B virus (HBV) is a small enveloped DNA virus that belongs to the *Hepadnaviridae* virus family. HBV may establish a chronic infection in the liver, which can, in turn, lead to cirrhosis and hepatocellular carcinoma (HCC). Although HBV has infected humans for at least 500 years[1], the virus was not discovered until 1966[2], and in 1970 Dane *et al*[3] identified the virus particle by electron microscopy. Since then, an antiviral therapy has been developed; these anti-HBV drugs are nucleos(t)ide analogs that can sufficiently suppress viral DNA load in most cases[4-9]. Moreover, vaccination programs have already been established to prevent HBV infection[10]. However, these are not sufficient to eradicate HBV. In fact, an estimated 257 million people are still chronically infected, and 887 thousand people die annually, primarily from the complications of HBV, which include cirrhosis and HCC[11-13].

Recently, RNAs, especially non-coding RNAs, have been revealed to have diverse functions[14]. We and others previously reported that viral RNAs not only work as templates for protein synthesis and viral DNA replication in the case of HBV but also exhibit biological functions involved in its pathogenesis[15,16]. In this context, even when HBV DNA is maintained at a relatively low level by nucleos(t)ide analogs, viral RNAs alone may harm the host, leading to cirrhosis or HCC. Thus, understanding the functions of HBV RNAs may act as a platform for the future development of HBV therapeutics. In this paper, we review current knowledge on the biological impact of HBV RNAs on host cells.

THE PROCESS OF HBV-RNA TRANSCRIPTION

The HBV genome has four overlapping open reading frames: 3.5 kb pre-C/C or pre-genomic RNA (pgRNA), 2.4 kb pre-S, and 0.7 kb X mRNA (Figure 1). Viral particles with a 3.2-kb-long partially double-stranded relaxed circular DNA (rcDNA) genome invade the cell through the sodium taurocholate co-transporting polypeptide (NTCP) receptor. After un-coating of surface antigen, the core particles transport the genome to the hepatocyte nucleus. Then, covalently closed circular DNA (cccDNA) is molded from rcDNA. The cccDNA plays a role as a template in the transcription of HBV RNA (Figure 2)[17].

The viral genes are transcribed by the cellular RNA polymerase II from cccDNA. Two enhancers designated enhancer I (EnhI) and enhancer II (EnhII) have been identified in the HBV genome, which drive and regulate the expression of the complete viral transcripts[18]. Moreover, recently, various host proteins were revealed to be involved in the process of HBV RNA transcription from cccDNA, and the most representative host proteins are structural maintenance of chromosomes (Smc) proteins Smc5 and Smc6. Because Smc5/6 inhibit HBV RNA transcription from cccDNA, the efficient transcription of HBV RNA from cccDNA requires the degradation of Smc5/6. HBV regulatory protein X (HBx) hijacks the host Cullin 4-ROC1 RING E3 ubiquitin ligase (CRL4) complex to target Smc5/6 co-localized with nuclear domain 10 (ND10) for ubiquitination, which, in turn, promotes HBV transcription[19-21]. Thus, the existence of HBV RNAs means the degradation of Smc5/6. Because Smc5/6 is related to DNA repair[22], this degradation may eventually lead to carcinogenesis. Therefore, this ubiquitination pathway has strong potential as a novel therapeutic target in interventions for HBV pathogenesis.

HBV RNAS MAY DEREGULATE THE FUNCTION OF HOST MICRO RNAS

MicroRNAs (miRNAs) are short, single-stranded, non-coding RNAs. Mature miRNAs are recruited into the Ago2-related RNA-induced silencing complex (RISC) and act to suppress the gene expression of target mRNAs. Depending on the target mRNA, miRNAs are responsible for various biological functions[23]. Recent studies have shown that HBV RNAs have several regions complementary to miRNAs, and act as miRNA sponges to upregulate the expression of miRNA targets; this results in the induction of HBV pathogenesis[15,24]. A list of miRNAs that could be trapped by HBV RNAs and may be involved in HBV pathogenesis is shown in Figure 3. In the following paragraphs, we discuss the potential biological roles of miRNAs in HBV pathogenesis.

PROMOTING VIRAL REPLICATION BY HBV RNAS

Although our knowledge of the direct relationship between HBV RNAs and viral replications are limited, HBV RNAs may promote viral replication *via* sequestering

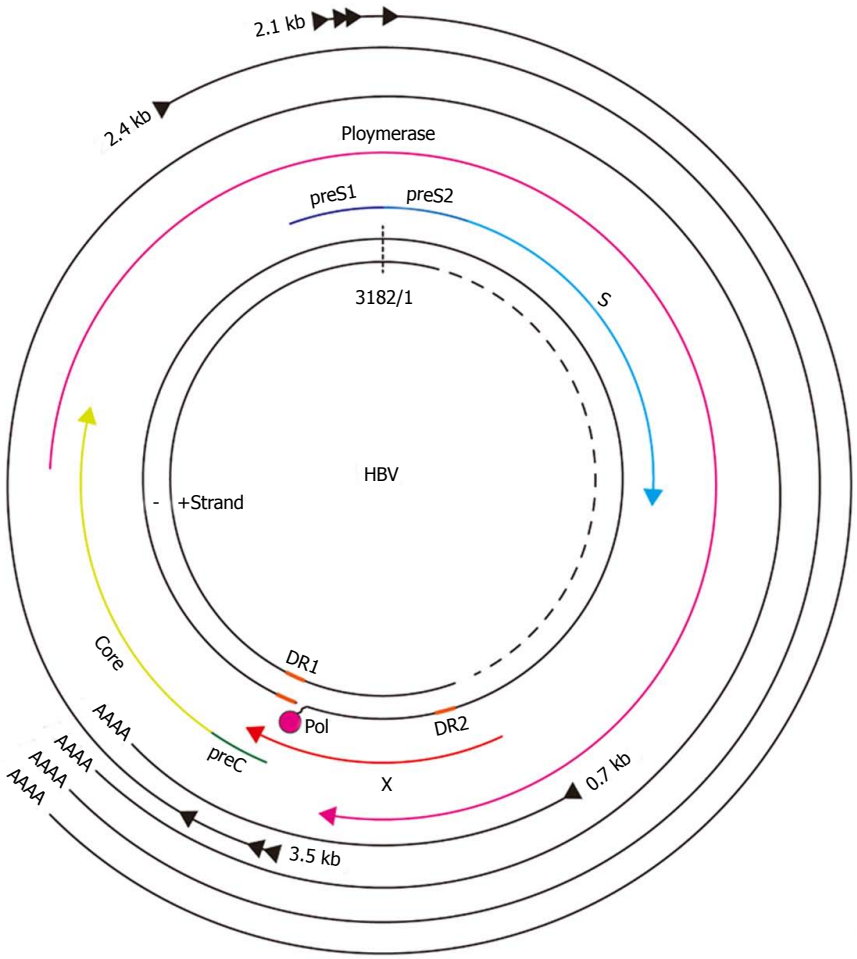

Figure 1 Hepatitis B virus genome structure. The 3.2-kb partially double-stranded relaxed circular DNA genome of the hepatitis B virus (HBV) is shown in the center. The surrounding colored arrows indicate the locations of the four overlapping open-reading frames. The black arrows show the viral mRNA. Pol: Polymerase; DR1: Direct repeat 1; DR2: Direct repeat 2.

cellular miRNAs, such as miR-122 and miR-15 family[25,26].

miR-122

miR-122 is highly and specifically expressed in hepatocytes. It plays multiple roles in the control of lipid metabolism, iron homeostasis, and the circadian rhythm and has anti-inflammatory and anti-tumorigenic functions[27-31]. The expression level of miR-122 is decreased in HBV-producing cells and in liver tissue from chronic hepatitis B patients[25,32,33]. Furthermore, there is an inverse correlation between miR-122 expression level and HBV replication[32]. Previously, it was found that the expression levels of pri-miR-122 and pre-miR122, the precursors of miR-122, were not decreased in HBV-positive HCC tissues and cells compared to normal liver tissue and cells[25,34,35]. Therefore, the downregulation of mature miR-122 expression is thought to be the result of binding to a conserved sequence at the 3' end of all HBV transcripts following degradation. Although the precise mechanisms remain to be clarified, viral non-coding RNAs may play a critical role modulating the turnover of host miRNAs through the degradation of target miRNAs[36-40].

miR-122 negatively regulates HBV replication. It has been reported that one possible mechanism mediating the negative regulation of HBV replication by miR-122 depends on the expression level of cyclin G1, a target of miR-122. Decreased expression or function of miR-122 would result in the suppression of p53 through upregulation of cyclin G1 expression, which further increases HBV transcription by blocking specific binding of p53 to HBV enhancer elements[33].

miR-15 family

The miR-15 family is also reported to regulate HBV replication. For instance, HBV RNA can sequester miR-15a and miR-16-1, and overexpression of these miRNAs decreases viral replication. Although the direct molecular mechanism of miR-15 family members has not been fully elucidated, among the multiple targets of miR-15a and miR16-1, cyclin D1 is thought to be involved in the regulation. Specifically, the up-regulation of cyclin D1 was demonstrated to be required for HBV replication[26].

HBV-encoded miRNA (HBV-miR-3)

Yang *et al*[41] recently showed that HBV-encoded HBV-

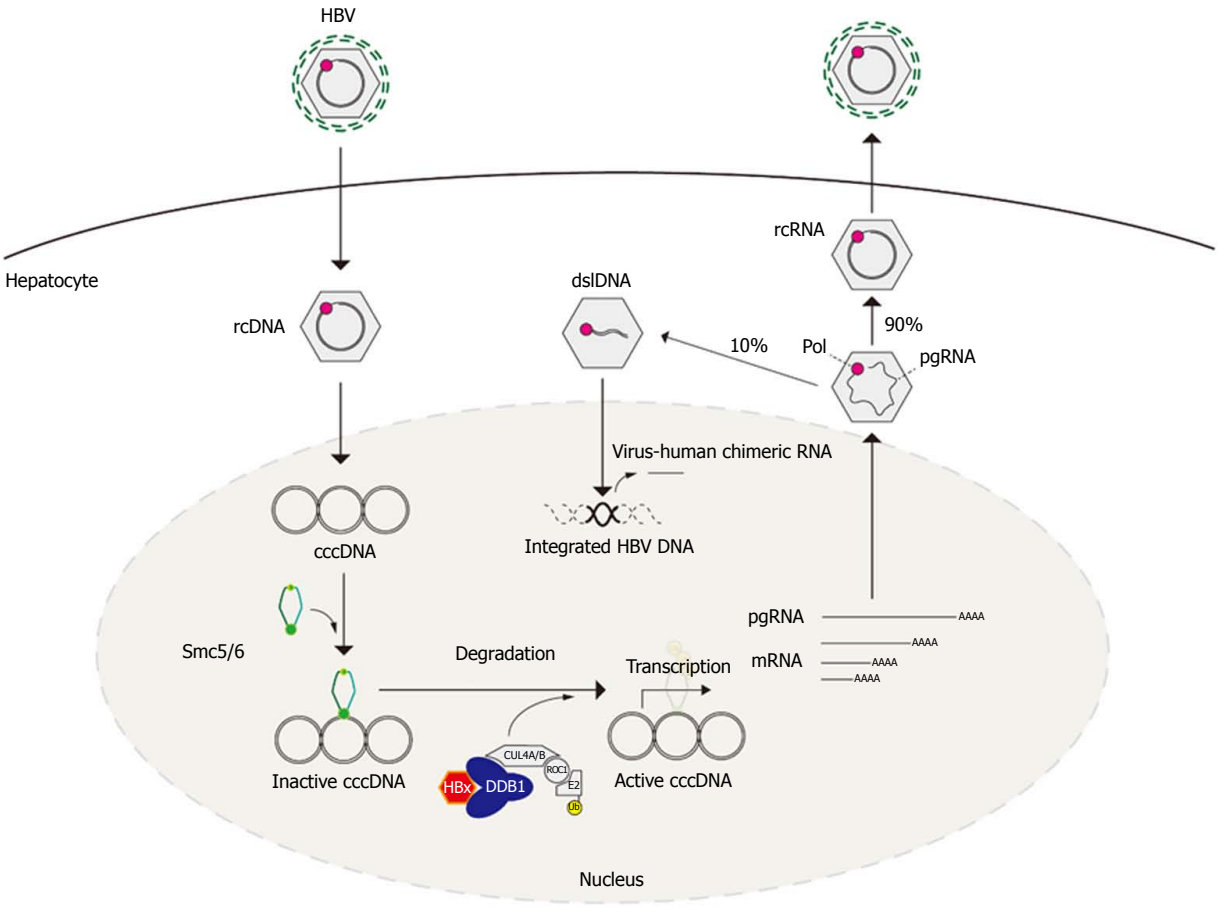

Figure 2 The life cycle of the hepatitis B virus. Hepatitis B virus (HBV) virions infect hepatocytes, and then rcDNA enters the nucleus and is converted to covalently closed circular DNA (cccDNA). Structural maintenance of chromosomes 5 and 6 (Smc5/6) can silence cccDNA, but HBV regulatory protein X (HBx) hijacks the Cullin 4-ROC1 RING E3 ubiquitin ligase (CRL4) complexes by binding to damage-specific DNA-binding protein 1 (DDB1) to target Smc5/6 for ubiquitination. Smc5/6 is consequently degraded by the proteasome, and cccDNA can then be transcribed. Transcribed HBV pregenomic RNA (pgRNA) is co-packaged with reverse transcriptase in capsids and is normally (~90%) reverse-transcribed into rcDNA, while double stranded linear DNA (dslDNA) is rarely (10%) synthesized depending on the binding region of the RNA primer. dslDNA can be integrated into the host cell genome, and virus-human chimeric RNA can be transcribed from integrated HBV DNA. After reverse transcription, the mature nucleocapsids can either be secreted as virions or cycle to the nucleus to add to the cccDNA pool.

miR-3 was expressed in HBV-infected tissues and cells. The viral-derived miRNA targeted the 3.5-kb HBV transcript to reduce HBc protein and pgRNA/HBV-RI production. The inhibition of HBV replication was suggested to contribute to the development of persistent infection in chronic hepatitis B patients. However, there is insufficient direct evidence for this mechanism, and, therefore, further studies are warranted.

PROMOTING HEPATIC FIBROSIS BY HBV RNAS

Liver fibrosis underlies the majority of chronic liver diseases and is a precursor to cirrhosis and HCC. The cycle of liver damage and repair leads to the deposition of extracellular matrix proteins and the development of fibrosis. Some miRNAs, such as miR-21, miR-221/222 and miR-181b, cause liver fibrosis through deregulation of the transforming growth factor-β (TGF-β) or nuclear factor-κB (NF-κB) pathways[42-44]. On the other hand, miR-29b, miR-101, miR-122, and miR-214-3p inhibit fibrosis by blocking collagen synthesis or the TGF-β pathway[45-48]. Among these miRNAs, miR-122 was reported to have complementary lesion(s) in HBV RNAs.

As previously mentioned, miR-122 is highly expressed in the healthy liver, but is downregulated in HBV-infected livers via sequestration by HBV RNA. This change in miR-122 expression leads to the development of liver fibrosis through the activation of collagen synthesis *via* the TGF-β pathway[47].

PROMOTION OF CARCINOGENESIS BY HBV RNAS

HBV is the leading risk factor for the development of HCC worldwide. Many mechanisms have been reported to lead to the development of HCC, and one such mechanism involves the sequestration of host miRNAs by HBV RNA.

miR-122

Decreased miR-122 levels resulted in increased pituitary

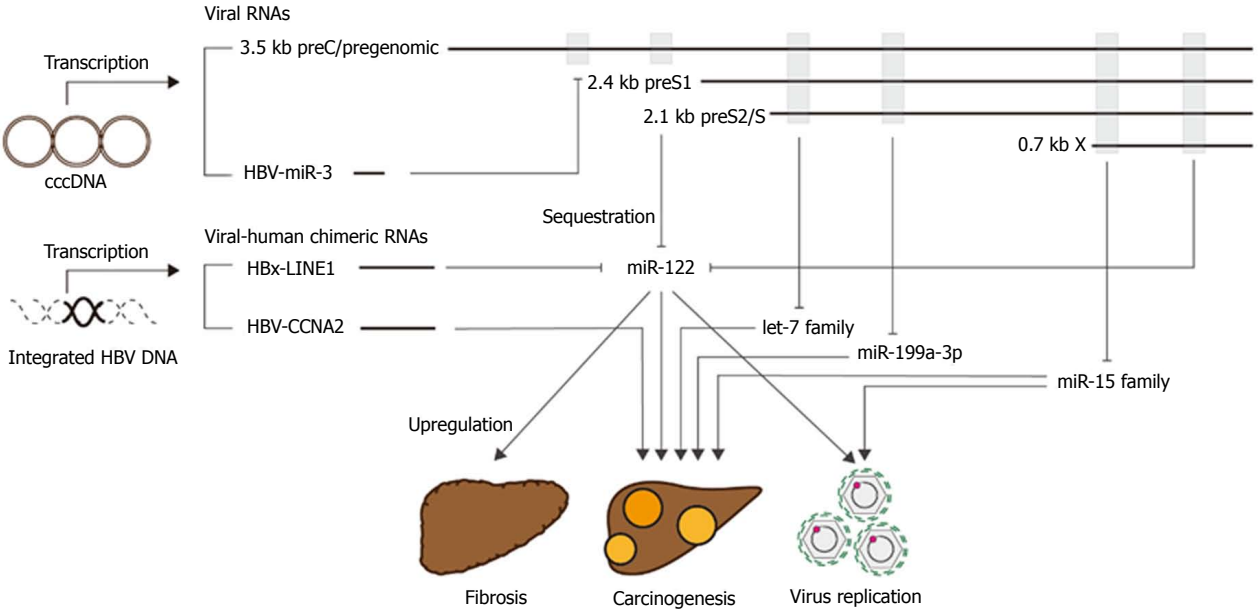

Figure 3 Hepatitis B virus-related RNAs alone have diverse effects on the host. Hepatitis B virus (HBV) mRNAs and HBV-miR-3 are transcribed from cccDNA, while viral-human chimeric RNAs are transcribed from integrated HBV DNA. These RNAs, except HBV-CCNA2, have complementary lesion(s) to cellular micro RNAs (miRNAs) and act as miRNA sponges, in turn triggering upregulated expression of the miRNA target and resulting in the induction of HBV pathogenesis. HBV-CCNA2 promotes tumor development through the newly synthesized chimeric transcript, which has new splice sites in the pre-mRNA produced by viral DNA integration.

tumor transforming gene 1 (PTTG1)-binding factor (PBF) expression, which enhanced the proliferation and invasiveness of HCC *in vitro* and tumorigenicity *in vivo*, through PBF-mediated activation of the PTTG1 transcription factor[25]. The possible contribution of these mechanisms to HBV-related carcinogenesis should be further examined in studies on human samples.

let-7 family

miRNAs in the let-7 family are classified as putative tumor suppressor miRNAs. The expression level of this family of miRNAs is often decreased in human cancers, including HCC, and promotes transformation by suppressing oncogenic targets, such as LIN28B, HMGA2 and c-Myc. Studies conducted by our group and others found that let-7 family miRNAs (*e.g.*, let-7g and let-7a) could be sequestered by HBV-RNA[15,24]. Furthermore, we demonstrated that this functional downregulation could lead to the promotion of tumorigenesis.

miR-199a-3p

miR-199a-3p is also involved in carcinogenesis and contributes to the malignant potential of HCC. Indeed, downregulation of miR-199a-3p correlated with poor HCC patient survival[49]. This miRNA targets mammalian target of rapamycin (mTOR) and c-Met in HCC cells. The restoration of miR-199a-3p levels in HCC cells resulted in G(1)-phase cell cycle arrest, decreased invasive capability, enhanced susceptibility to hypoxia, and increased sensitivity to doxorubicin-induced apoptosis.

miR-15a

miR-15a can be sponged off by HBV mRNAs. One of the proposed targets of miR-15a is Smad7, an inhibitor of the TGF-β pathway. Thus, HBV mRNA can interfere with TGF-β signaling by upregulating Smad7 expression, which obstructs TGF-β-induced apoptosis and promotes tumor development[50].

RNAS PRODUCED FROM INTEGRATED HBV DNA MAY PROMOTE CARCINOGENESIS

HBV DNA can integrate into host chromosomes at various locations. Integrated HBV DNA lacks the ability to transcribe pgRNA because HBV double-stranded linear DNA is only ~16 nt longer than the length of the genome, making it too short to transcribe pgRNA. Despite this, integrated HBV DNA levels correlate with the development of HCC. Indeed, the majority of HBV-related HCCs contain at least one HBV genome integration site[51]. While the mechanism of carcinogenesis induced by the integration of the HBV genome has been explained in several ways, virus-related RNAs from the integration sites are definitely involved.

HBx-long interspersed nuclear element 1

HBV DNA integration often occurs within or near repetitive, non-coding sequences, such as long interspersed nuclear element 1 (LINEs) and short interspersed nuclear elements (SINEs)[52]. By applying Viral-Fusion-Seq to detect possible fusions between viral and human sequences[53], a viral-human hybrid RNA transcript called HBx-LINE1 was identified in HBV-related HCCs[54]. The presence of this long non-coding RNA, a fusion of the

human LINE1 and HBx genes, was correlated with poor prognosis in HCC patients[54].

HBx-LINE1 contains six binding sites for miR-122, which enable the chimeric HBx-LINE1 transcript to act as a molecular sponge for miR-122. This sequestration leads to an increase in hepatic cell β-catenin signaling, a decrease in E-cadherin levels, increased cell migration, and significant mouse liver injury, leading to HCC[35]. Therefore, HBx-LINE1 is a potential therapeutic target and prognostic biomarker for HCC. While this is an interesting result, further studies are needed to uncover the precise mechanism of oncogenesis.

HBV-cyclin A2

Cyclin A2 (CCNA2) is a cell cycle regulatory protein that acts as a regulatory subunit of cyclin-dependent kinase[55]. Integration of HBV into the CCNA2 gene has been observed in HBV-positive HCCs[56]. The integration site is intron 2 of CCNA2, which results in the formation of a new splice site in the pre-mRNA. This new splice site leads to the formation of a 177-bp in-frame pseudo-exon and produces a novel and recurrent HBV-CCNA2 fusion transcript, A2S[56]. Disruption of the destruction box of A2S causes A2S to become non-degradable; however, the function enhancing cell cycle progression of CCNA2 is retained, which demonstrates its potential role in hepatocarcinogenesis.

FUTURE STRATEGY

In this review, we summarized current knowledge on the roles of HBV RNAs, including viral replication, promotion of liver fibrosis, and carcinogenesis, in HBV-related pathogenesis. Specifically, we discussed how HBV RNAs deregulate miRNA function and lead to the synthesis of host-viral fusion RNA from integration sites. However, HBV RNAs may still have other, as yet unknown biological functions, such as deregulating host protein function or long non-coding RNA function through direct interactions or associations. Therefore, further studies are needed to fully elucidate the biological roles of HBV RNAs.

Certainly, anti-HBV therapeutics must focus on the elimination of HBV RNAs; however, no such therapeutic is currently available. The ultimate therapeutic goal is to destroy cccDNA. While gene-editing approaches, such as those focused on the CRISPR/Cas9 system, may be reasonable for directly targeting cccDNA, further studies are necessary to identify strategies to maximize positive effects and minimize toxicity[17,57]. In the meantime, transcriptional silencing of cccDNA may be a practical approach to attenuate HBV-related pathogenesis. For this purpose, a full understanding of HBV transcriptional control and HBV RNA-mediated pathogenesis is urgently needed.

CONCLUSION

HBV RNAs are not only templates for protein synthesis and viral DNA replication but also exhibit biological functions that play a role in pathogenesis. Because current therapies are unable to solve this problem, novel therapeutic agents that target the cccDNA itself, or inhibit its transcription, are strongly warranted.

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ORIGINAL ARTICLE

Basic Study

Multiple cytokine profiling in serum for early detection of gastric cancer

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Abstract

AIM

To investigate the value of multiparameter joint analysis in the early diagnosis of gastric cancer (GC) in clinical practice.

METHODS

Concentrations of CEA, CA724 and three kinds of cytokines (TNF-α, IL-6 and IL-8) in 176 GC patients, 117 atypical hyperplasia patients, and 204 healthy control individuals were used for building the diagnostic model, then 58 GC patients, 41 atypical hyperplasia patients, and 66 healthy control individuals were enrolled independently. The joints of the indicators were analyzed by binary logistic regression analysis method.

RESULTS

For discriminating the healthy control group and the GC group, IL-6 had the best diagnostic value, and the area under curve (AUC) of joint analysis was 0.95 (0.93-0.97). For the early stage and advanced stage GC, the AUC were 0.95 (0.92-0.98) and 0.95 (0.92-0.97). For discriminating the atypical hyperplasia group and GC group, CA724 had the best diagnostic value, and the AUC of joint analysis was 0.97 (0.95-0.99). For the early stage and advanced stage GC groups, the AUC were 0.98 (0.96-0.99) and 0.96 (0.94-0.98). After evaluation, for discriminating the GC, early stage GC and advanced cancer group from the healthy control group, the diagnostic sensitivity was 89.66%, 84.21% and 92.31%, respectively, and the specificity was 92.42%, 90.91% and 90.91%. For discriminating the GC, early stage GC and advanced cancer groups from the atypical hyperplasia group, the diagnostic sensitivity was 87.93%, 78.95% and 92.31%, respectively, and the specificity was 87.80%, 85.37% and 90.24%.

CONCLUSION

We have built a diagnostic model including CEA, CA724, IL-6, IL-8, and TNF-α. It may provide potential assistance as a screening method for the early detection of GC.

Key words: Gastric cancer; Atypical hyperplasia; Serum; Cytokine; Early detection

Core tip: We aimed to use multiparameter joint analysis for improving sensitivity and specificity for detection of gastric cancer. By combining CEA, CA724, IL-6, IL-8 and TNF-α, we built a diagnostic model, which may provide potential assistance as a screening method for the early detection of gastric cancer.

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INTRODUCTION

Gastric cancer is a kind of malignant tumor derived from gastric mucosal epithelial cells[1-3]. It is the fourth most common malignancy worldwide, and it ranks second in terms of the number of deaths[4]. In China, gastric cancer is one of the most malignant tumors, with high morbidity and mortality[5]. Deaths from gastric cancer account for approximately 25% to 30% of the deaths from all cancer types[6]. Its pathogenesis involves aging of the body, eating habits and psychological factors[7-9]. In recent years, more stress, poor diet and overwork have been shown to have greater influence on the incidence of gastric cancer. The occurrence and development of gastric cancer is a multistep process[10]. In current clinical practice, the main treatment for gastric cancer is surgery. The 5-year survival rate is very low[11]; however, if the gastric cancer is detected at an early stage, the 5-year survival rate can be as high as 90%[12]. Early diagnosis and treatment of gastric cancer is extremely important for gastric cancer patients.

At present, many methods of diagnosing gastric cancer are used in scientific research and clinical practice[13]. Serologic biomarkers are important detection methods. In early gastric cancer, the tumor markers [such as carcinoembryonic antigen (CEA) and cancer antigen (CA)724] are increased to some extent in the blood. The levels of these markers have been used as important indicators in gastric cancer screening, early diagnosis and prognosis evaluation[14]. However, no specific tumor marker has been found at present. Diagnosis based on a single tumor marker has some limitations[15]. The detection rate of gastric cancer is still very low.

Cytokines are small molecules secreted by cells in response to various stimuli, and they are involved in biological processes, through their binding to specific receptors on target cells[16]. Many studies have demonstrated that cytokine production and cellular immune function are important regulatory factors in the development of tumors[17-19]. As multifunctional molecules, these inflammatory factors not only directly damage tumor cells but also act as important mediators in the killing of tumor cells by mononuclear cells. The relationship of cytokines and gastric cancer provides a new direction for exploring the pathological mechanism of gastric cancer and may also provide a potential means of diagnosing and treating gastric cancer in the clinical setting.

Studies have confirmed that patients with cancer usually have defects in their immune function, especially having cellular immune dysfunction. TNF-α, IL-6 and IL-8 are important mediators of the inflammatory response and a series of other pathophysiological processes *in vivo*[20-22]. Their value in the diagnosis of gastric cancer has been evaluated, although their diagnostic value in combination with conventional biomarkers, such as CEA and CA724, has not been studied.

In this study, we first evaluated the diagnostic value of CEA, CA724 and three cytokines (TNF-α, IL-6 and IL-8) for gastric cancer. Then, we analyzed the combinations of the conventional biomarkers with the cytokines by using binary logistic regression. Our aim was to use the multiparameter joint analysis to improve diagnostic sensitivity and specificity and to provide a novel potential method for the early diagnosis of gastric cancer in clinical practice.

MATERIALS AND METHODS

Samples enrolled

Written consent was obtained. The study was reviewed and approved by the Institutional Review Board of the Affiliated Tumor Hospital of Zhengzhou University. This study was conducted from January 2015 to December 2016. There were 176 gastric cancer patients enrolled in our study (63 early-stage and 113 advanced-stage patients). The stages were confirmed by pathological examination. All the gastric cancer patients were enrolled before surgery, chemotherapy, radiotherapy and immunotherapy. In addition, 117 atypical hyperplasia patients were enrolled. The examination results were confirmed by gastroscopy and pathological examination.

Finally, 204 healthy control individuals were also enrolled. The healthy controls were without obvious disease, and the results of the basic tests were checked by B-mode ultrasound and CT examination, including of the heart, brain, kidney and other important organs. After building the diagnostic model, 58 gastric cancer patients (19 early-stage and 39 advanced-stage patients), 41 atypical hyperplasia patients, and 66 healthy control individuals were independently enrolled.

Serum collection and detection equipment

After the collection of whole blood samples, the tubes were centrifuged for 7 min at 3500 r/min and immediately stored at -80 ℃. The CEA and CA724 levels were detected by the Roche Modular E170 automatic electrochemiluminescence immunoassay analyzer. The reagents, standards and controls were purchased from Roche. The serum levels of IL-6, IL-8 and TNF-α were detected by Luminex 200, and the detection kits were purchased from Millipore.

Serum concentrations of IL-6, IL-8 and TNF-α

Serum samples from the cancer group and the control group were stored in a freezer at -80 ℃. Before performing the experiment, the serum samples were thawed, and 100 μL of serum was transferred from each sample to centrifuge tubes. The reagents were allowed to equilibrate to room temperature at 25 ℃, and wash buffer was diluted 10 times with deionized water.

The serum concentrations of IL-6, IL-8 and TNF-α were determined by the following protocol. First, 200 μL of assay buffer was added to each reaction well in a 96-well plate. After sealing, the solution was mixed thoroughly on a horizontal shaker, and the assay buffer was vacuumed and then blotted on the bottom of the plate. Second, 25 μL of each standard or control was added to the appropriate wells, and 25 μL of assay buffer was also added to each well, followed by the addition of 25 μL of serum matrix diluent to the standard and control wells. Third, after mixing the microspheres well, 25 μL of hybrid microspheres were added to each well, and the plate was covered with sealing film and foil, before incubation overnight at 4 ℃ on a horizontal shaker. Fourth, after washing, 25 μL of the detection antibody was added to each well and incubated for 1 h at room temperature. Then, 25 μL of streptavidin-PE was added to each well and incubated for 30 min at room temperature. Fifth, after washing, the 96-well plate was placed in the Luminex reading instrument, and the levels were calculated according to the standard curve.

Statistical methods

SPSS 21.0 statistical software was used to analyze the data. The serum levels of CEA, CA724, IL-6, IL-8 and TNF-α in the different groups were compared by one-way ANOVA. The diagnostic value was evaluated by the area under the curve (AUC) of the receiver operator characteristic (ROC) curve, and the cutoff value was determined by the Youden index. The combinations of the indicators were analyzed by the binary logistic regression analysis method[23]. $P < 0.05$ indicated statistical significance.

RESULTS

Comparison of CEA, CA724, IL-6, IL-8 and TNF-α levels in the three groups

As shown in Figure 1, the concentrations of CEA, CA724, IL-6, IL-8 and TNF-α in the healthy control group, the atypical hyperplasia group and the gastric cancer group were compared. As shown in Figure 1A, the concentrations of IL-6 in the healthy control, atypical hyperplasia and gastric cancer groups were 10.05 (6.47, 18.26), 50.17 (23.93, 110.40) and 63.96 (38.93, 139.10), respectively. The concentrations of IL-8 were 0.48 (0.07, 1.17), 0.85 (0.33, 2.44) and 1.80 (0.11, 6.28), respectively (Figure 1B). The concentrations of TNF-α were 5.49 (4.16, 7.21), 6.73 (5.31, 8.27) and 10.20 (5.88, 16.41), respectively (Figure 1C). The concentrations of CEA were 1.53 (0.91, 2.26), 1.51 (1.15, 2.05) and 2.35 (1.12, 5.22), respectively (Figure 1D). The concentrations of CA724 were 2.02 (1.15, 4.30), 2.21 (1.02, 3.41) and 4.03 (1.52, 11.62), respectively (Figure 1E). The concentrations of IL-6, IL-8, TNF-α, CEA and CA724 in the atypical hyperplasia group and gastric cancer group were significantly different from those in the healthy control group. The concentrations of IL-6, IL-8, TNF-α and CA724 in the gastric cancer group were significantly different compared to those of the atypical hyperplasia group.

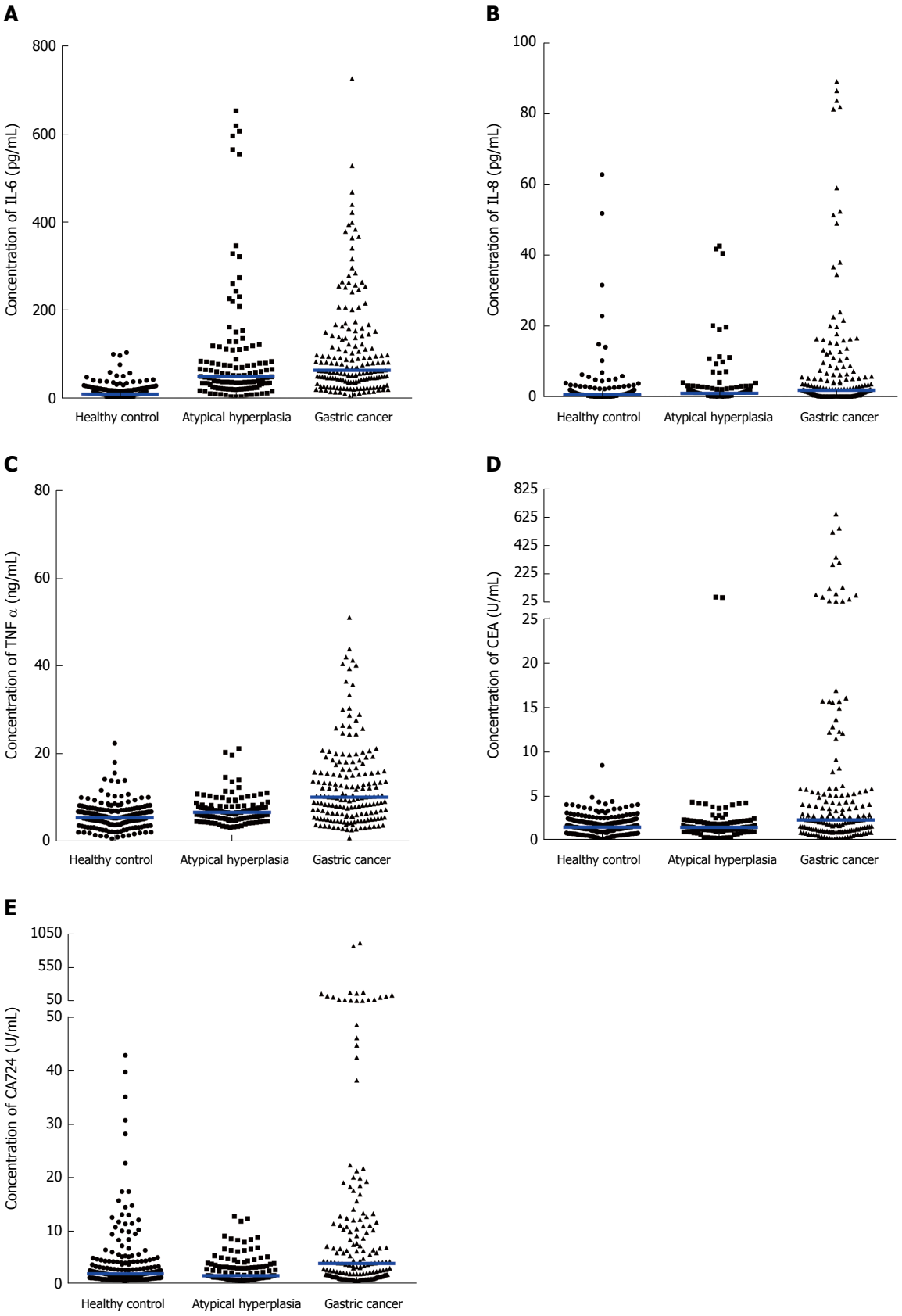

Figure 1 Comparison of carcinoembryonic antigen, CA724, IL-6, IL-8 and TNF-α in the three groups. A: IL-6; B: IL-8; C: TNF-α; D: CEA; E: CA724. CA: Cancer antigen; CEA: Carcinoembryonic antigen.

Table 1 Diagnostic value of the five indicators for discriminating the healthy control group and the gastric cancer group

Indicator	AUC	95%CI of AUC	Cutoff value	Sensitivity, %	Specificity, %
IL-6	0.92	0.91-0.94	20.31	92.05	78.92
IL-8	0.65	0.60-0.71	1.45	55.68	79.41
TNF-α	0.76	0.71-0.81	7.82	65.91	82.84
CEA	0.65	0.60-0.71	3.45	36.36	92.65
CA724	0.64	0.58-0.70	5.80	40.91	84.34

AUC: Area under curve; CA: Cancer antigen; CEA: Carcinoembryonic antigen; CI: Confidence interval; IL: Interleukin; TNF: Tumor necrosis factor.

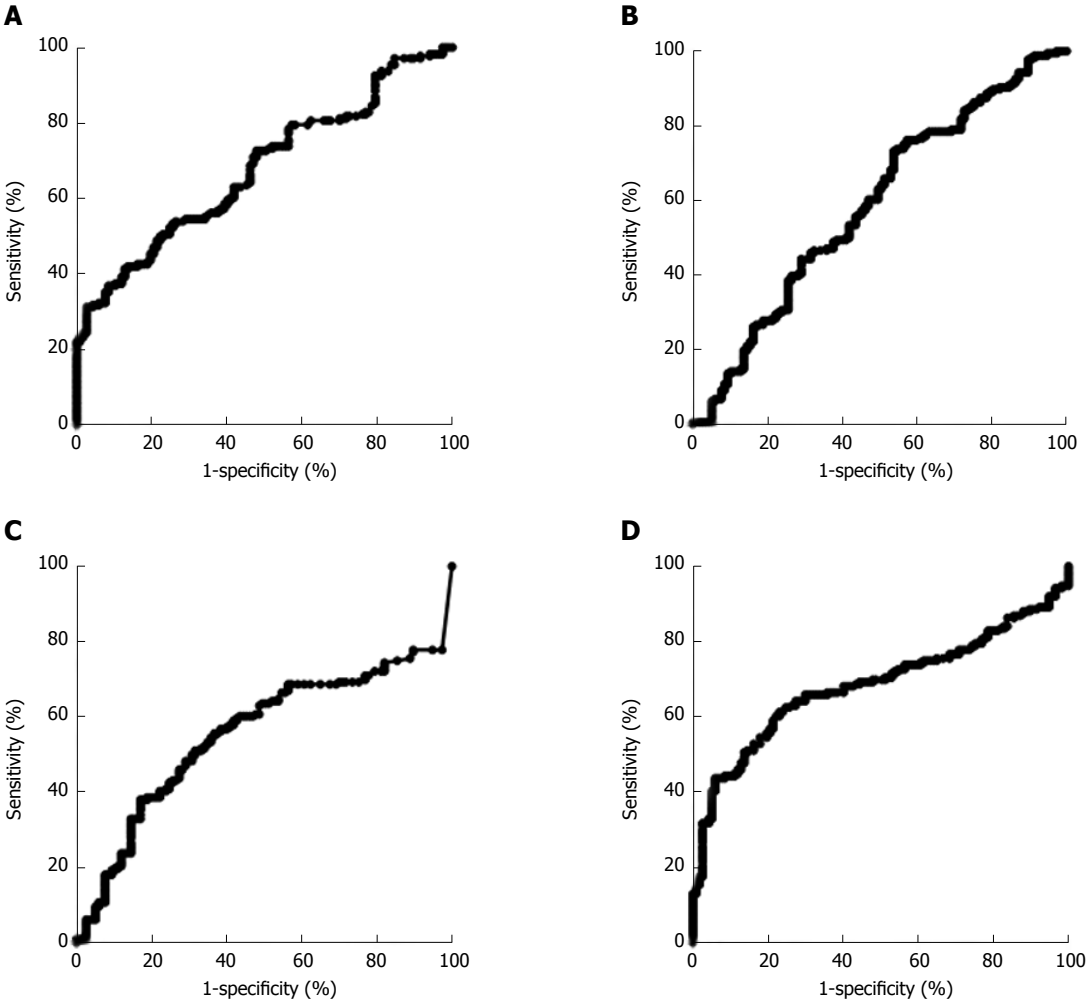

Figure 2 Diagnostic value of IL-6, IL-8, TNF-α and CA724 for discriminating the atypical hyperplasia group and gastric cancer group. A: IL-6; B: IL-8; C: TNF-α; D: CA724. CA: Cancer antigen; CEA: Carcinoembryonic antigen.

Diagnostic value of the concentrations of CEA, CA724, IL-6, IL-8 and TNF-α for the detection of gastric cancer

As shown in Table 1, when the concentrations of CEA, CA724, IL-6, IL-8 and TNF-α were used alone to discriminate between the healthy control group and the gastric cancer group, the AUCs of the five indicators ranged from 0.64 to 0.93. The concentration of IL-6 had the best diagnostic value for discriminating between the healthy control group and the gastric cancer group. When the cutoff value was 20.31 pg/mL, the sensitivity and specificity were 92.05% and 78.92%, respectively. For the two conventional biomarkers, CEA and CA724, the AUCs were 0.65 (0.60-0.71) and 0.64 (0.58-0.70), respectively. To discriminate between the atypical hyperplasia group and the gastric cancer group, as shown in Figure 2A, the conventional biomarker CA724 had the best diagnostic value, with an AUC of 0.68 (0.62-0.74). When the cutoff value was 9.13 U/mL, the sensitivity and specificity were 31.25% and 97.44%, respectively. The three cytokines, IL-6, IL-8 and TNF-α, showed poorer diagnostic values, and their AUCs were 0.59 (0.52-0.66), 0.55 (0.49-0.63) and 0.68 (0.62-0.74),

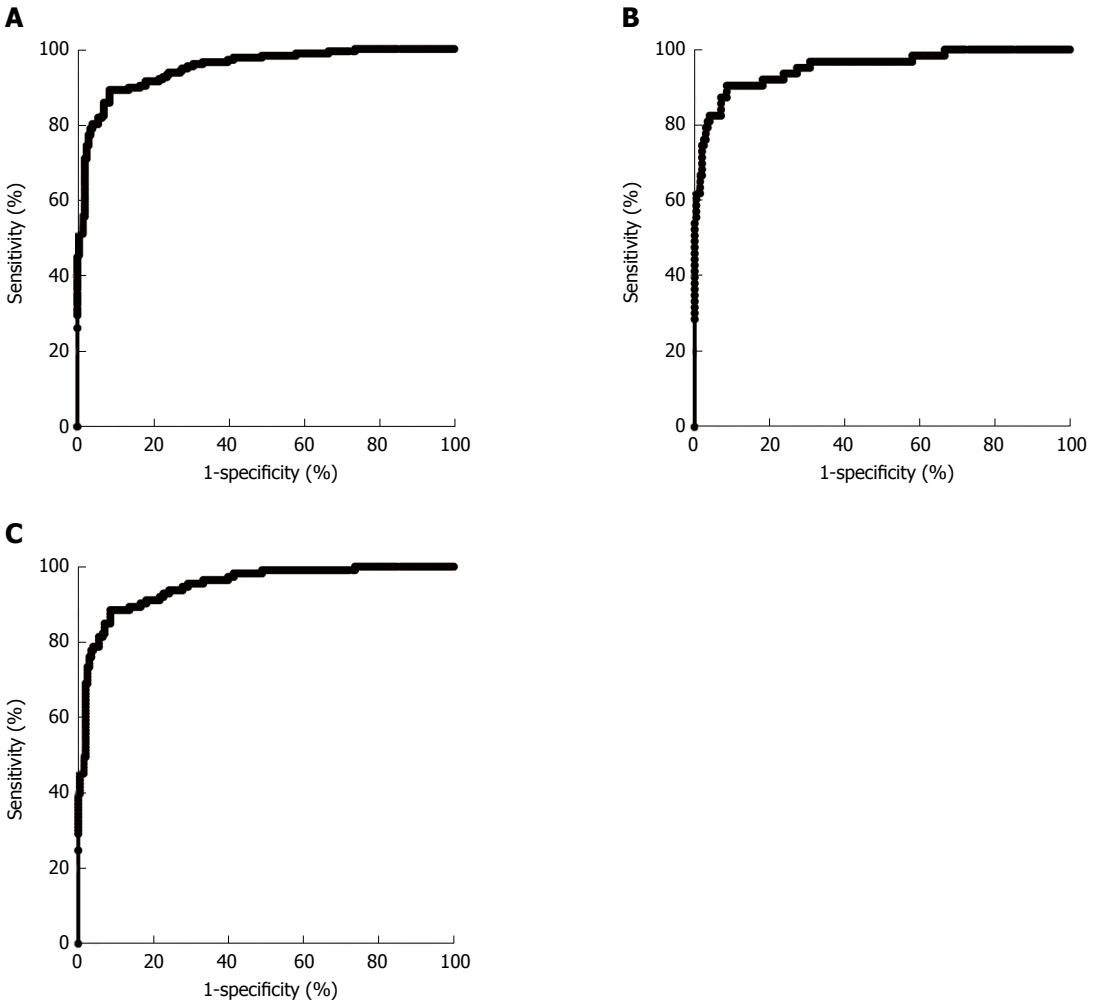

Figure 3 Joint analysis of CEA, CA724, IL-6, IL-8 and TNF-α for discriminating the healthy control group and gastric cancer group. A: Healthy control group *vs* gastric cancer group; B: Healthy control group *vs* early stage gastric cancer group; C: Healthy control group *vs* advanced stage gastric cancer group. CA: Cancer antigen; CEA: Carcinoembryonic antigen.

respectively (Figure 2B-D).

Joint analysis of the diagnostic value of the concentrations of CEA, CA724, IL-6, IL-8 and TNF-α for the detection of gastric cancer

After evaluating the diagnostic value of the concentrations of CEA, CA724, IL-6, IL-8 and TNF-α separately, binary logistic regression was used to analyze the indicators jointly. As shown in Figure 3A, for discriminating between the healthy control group and the gastric cancer group, the AUC was 0.95 (0.93- 0.97). For early-stage gastric cancer, the AUC was 0.95 (0.92- 0.98), and for advanced-stage gastric cancer, it was 0.95 (0.92- 0.97), as shown in Figure 3B and 3C. For discriminating between the healthy control group and the gastric cancer group, our joint analysis method showed similar diagnostic values for early-stage and advanced-stage gastric cancer.

For discriminating between the atypical hyperplasia group and the gastric cancer group, four indicators, namely CA724, IL-6, IL-8 and TNF-α, were used in the joint analysis. As shown in Figure 4A, for discriminating between the atypical hyperplasia group and the gastric cancer group, the AUC was 0.97 (0.95-0.99). For early-stage gastric cancer, the AUC was 0.98 (0.96-0.99), and for advanced-stage gastric cancer, it was 0.96 (0.94-0.98), as shown in Figure 4B and 4C. For discriminating between the atypical hyperplasia group and the gastric cancer group, our joint analysis method also showed similar diagnostic values for early-stage and advanced-stage gastric cancer.

Validation of the joint analysis for the detection of gastric cancer

After building the diagnostic model, 58 gastric cancer patients (19 early-stage and 39 advanced-stage patients), 41 atypical hyperplasia patients, and 66 healthy control individuals were independently enrolled. Then, the diagnostic model including CEA, CA724, IL-6, IL-8 and TNF-α for discriminating between the healthy control group and the gastric cancer group and the diagnostic model including CA724, IL-6, IL-8 and TNF-α for discriminating between the atypical hyperplasia group and the gastric cancer group were evaluated. After evaluation, for discriminating between

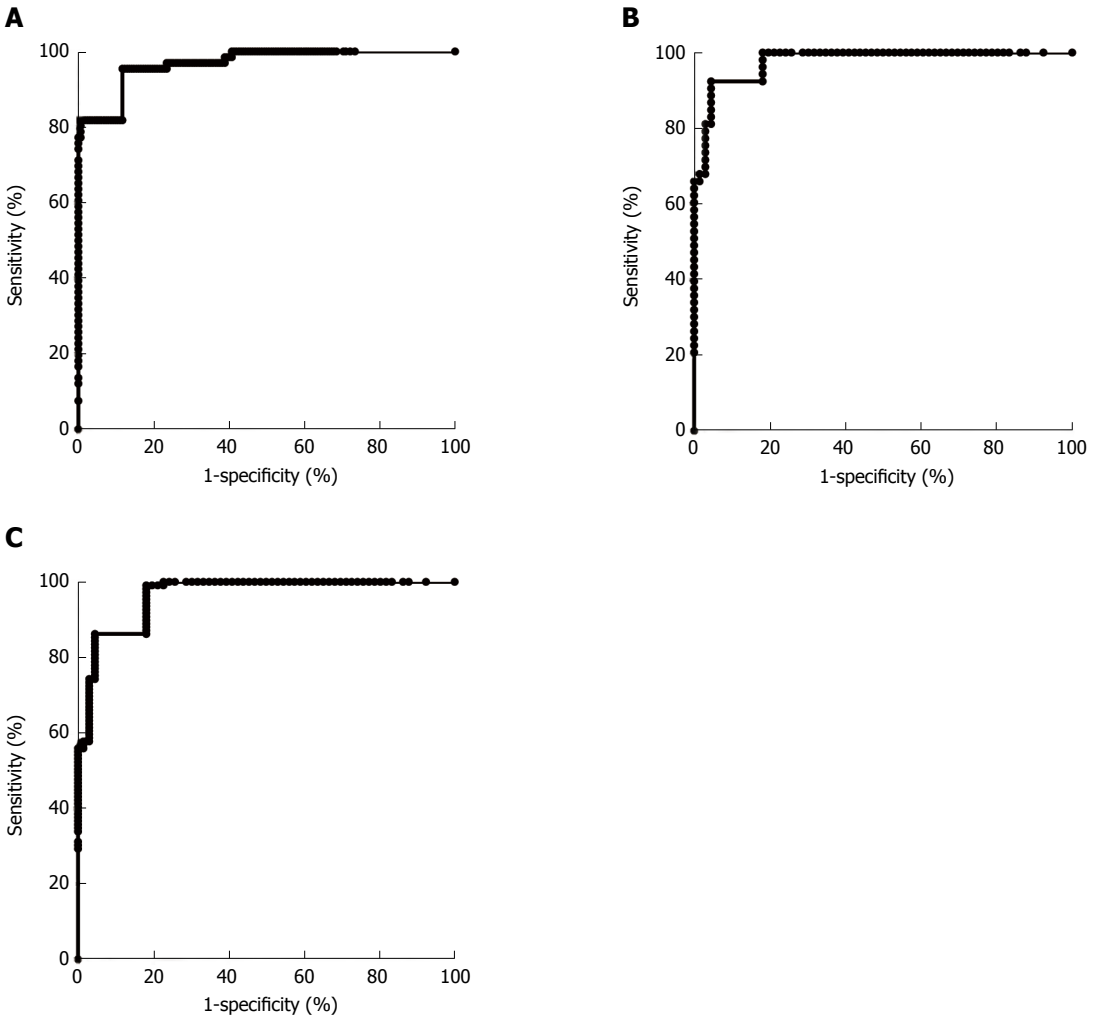

Figure 4 Joint analysis of IL-6, IL-8, TNF-α and CA724 for discriminating the atypical hyperplasia group and gastric cancer group. A: Atypical hyperplasia group *vs* gastric cancer group; B: Atypical hyperplasia group *vs* early stage gastric cancer group; C: Atypical hyperplasia group *vs* advanced stage gastric cancer group. CA: Cancer antigen; CEA: Carcinoembryonic antigen.

the healthy control group and the gastric cancer group, the early-stage gastric cancer group and the advanced-stage gastric cancer group, the diagnostic sensitivities were 89.66%, 84.21% and 92.31%, respectively. In addition, the specificities were 92.42%, 90.91% and 90.91%, respectively. For discriminating between the atypical hyperplasia group and the gastric cancer group, the early-stage gastric cancer group and the advanced-stage gastric cancer group, the diagnostic sensitivities were 87.93%, 78.95% and 92.31%, respectively. In addition, the specificities were 87.80%, 85.37% and 90.24%, respectively.

DISCUSSION

According to the estimates of the World Health Organization, nearly 7 million people die from tumors each year worldwide, and that number is increasing annually. Gastric cancer is one of the common malignant tumors that endanger human health. It causes the second highest number of cancer-related deaths. The occurrence and development of gastric cancer is a multistage process, involving multiple gene and molecular level changes. In the pregastric cancer stage there are precancerous lesions, most of which remain unchanged and a small part of which develop into cancer.

The Correa cascade is the most commonly recognized pattern of gastric carcinogenesis[24]. Because most gastrointestinal cancer has no obvious symptoms in the early stage, it cannot be detected in a timely manner; however, when clinical symptoms develop, it is often too late to effectively treat the cancer, resulting in low postoperative survival rates of patients with malignant tumors. Early detection is the key to improving the survival rate of patients and the cure rate[12]. Therefore, early detection of gastric cancer is crucial to the improvement of the treatment of gastric cancer.

CEA is a cell surface antigen. It is a tumor-associated antigen extracted from embryonic tissue and can be detected in a variety of body fluids. As one of the most common tumor markers, it is widely used as a diagnostic and monitoring index for various gastrointestinal tumors, especially gastric adenocarcinoma[25]. CA724 is a high molecular weight glycoprotein, and it is one of the best

tumor markers for the diagnosis of gastric cancer. It has high specificity for gastric cancer and has good applicability in digestive system malignant tumors[26]. The results of our study showed that the serum levels of CEA and CA724 in the gastric cancer group were significantly higher than those in the atypical hyperplasia and healthy control groups. The results were consistent with those of previous studies[27,28] and indicated that these markers have certain diagnostic value for gastric cancer.

The inflammation in cancer is a multifactorial process. Phagocytes are effector cells that initiate inflammation. They can use a variety of surface receptors to identify invading foreign microorganisms that they finally kill. In this process, activated phagocytes secrete a large number of pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α. The expression levels of these pro-inflammatory cytokines are significantly increased in inflammatory diseases.

As a very important immunosuppressive regulator, IL-8 is a cytokine secreted by fibroblasts, epithelial cells and mononuclear macrophages, and it plays an important role in the growth, differentiation or gene expression of many kinds of cells[29]. In gastric cancer patients, the expression levels of IL-8 are higher in the tumor tissue, serum and malignant effusion of the thoracic and abdominal cavity but lower in normal tissues and serum. In addition, IL-8 also plays an important role in the angiogenesis of gastric tumors. It can act on vascular endothelial cells, inducing large-scale proliferation of endothelial cells to promote angiogenesis[30]. In our experiment, the levels of IL-8 in patients with gastric diseases (gastric cancer group and atypical hyperplasia group) were significantly higher than that in the healthy control group. The results showed that IL-8 was highly expressed in patients with gastric cancer and gastric inflammatory diseases, which was consistent with the results of previous studies.

IL-6 has been demonstrated to play a role in tumor metastasis and tumor angiogenesis[31]. The IL-6 gene is active in many tumor tissues and peripheral blood vessels, and the secretion of various cytokines is increased. Numerous studies have demonstrated that it not only directly stimulates monocyte-derived macrophages and fibroblasts to secrete IL-6 but also that cancer cells can secrete a large amount of IL-1α to promote the proliferation of malignant cells in their own growth process[32]. The imbalance of IL-6 and its receptor affects the stability of the whole environment and leads to disordered immune function, which may induce tumors[33]. In our study, the level of IL-6 was significantly higher in gastric cancer patients than in atypical hyperplasia patients. Previous studies also found that tumors were associated with abnormal expression of IL-6.

TNF-α is a multifunctional cytokine produced by macrophages and activated T cells. It is involved in inducing an acute albumin reaction, activating neutrophils and lymphocytes, regulating the metabolic activity of tissues and promoting the release of other cytokines[11]. Studies have shown that TNF-α can kill a variety of tumor cells and enhance the body's anti-tumor action, but it can also promote the growth and metastasis of some tumors. It can cause tumor tissue hypoxia and vascular damage around the tumor, promoting the cytotoxic effect of natural killer cells and macrophages and enhancing the body's immunity, thereby inhibiting tumor growth[34]. When the level of TNF-α is abnormal, the patient's immune system is disordered, which triggers systemic cytotoxicity, resulting in escape of the tumor cells from host immune surveillance and allowing them to continue to grow[35]. In our study, the levels of TNF-α in the gastric cancer and atypical hyperplasia groups were significantly higher than in the healthy control group, suggesting that TNF-α may be closely related to the occurrence and development of gastric cancer. As an important regulator of inflammation, TNF-α may play a role in tumor-associated inflammatory processes, increasing the risk of inflammation-induced tumors. Our results were consistent with those of previous studies.

Although we have built a potential diagnostic model for the early detection of gastric cancer, there were still some limitations to our study. First, there were only three investigated in our study, and many other kinds of cytokines were excluded. Second, the Luminex 200 detection system may be too sensitive, resulting in a high degree of variance, which may have affected the results of our study. Third, the sample size of our study was relatively small, and the diagnostic model validation was only performed in a small cohort.

In conclusion, we have built a diagnostic model including the levels of CEA, CA724, IL-6, IL-8 and TNF-α. It may provide a potential screening method for the early detection of gastric cancer.

ARTICLE HIGHLIGHTS

Research background

Early diagnosis and treatment of gastric cancer (GC) is extremely important for GC; however, there is still no effective detection method for the early detection of GC.

Research motivation

Many studies have demonstrated that the joint analysis of a panel of indicators may improve the diagnostic value for kinds of cancers. Cytokines have also been demonstrated to play important roles in the development of cancer.

Research methods

Concentrations of carcinoembryonic antigen (CEA), cancer antigen (CA)724, TNF-α, IL-6 and IL-8 in 176 GC patients, 117 atypical hyperplasia patients and 204 healthy controls were used for building the model; then, 58 GC patients, 41 atypical hyperplasia patients and 66 healthy controls were used for validation. The joints of the indicators were analyzed by binary logistic regression analysis method.

Research results

For discriminating the GC, early-stage GC and advanced cancer patients from the healthy control group, the diagnostic sensitivity was 89.66%, 84.21% and 92.31%, respectively. The specificity was 92.42%, 90.91% and 90.91%, respectively. For discriminating the GC, early stage GC and advanced cancer

patients from the atypical hyperplasia group, the diagnostic sensitivity was 87.93%, 78.95% and 92.31%, respectively. The specificity was 87.80%, 85.37% and 90.24%, respectively.

Research conclusions

We have built a diagnostic model including CEA, CA724, IL-6, IL-8 and TNF-α, and it may represent a potential assistant screening method for the early detection of GC.

Research perspectives

Our study provides a simple, effective and noninvasive detection method for the assistant detection of GC. In the future study, multicenter and larger sample size design should be included to validate the diagnostic value.

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ORIGINAL ARTICLE

Retrospective Study

Magnetic resonance imaging and Crohn's disease endoscopic index of severity: Correlations and concordance

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Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

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Abstract

AIM

To examine the correlation between magnetic resonance imaging (MRI) and endoscopic index of severity (CDEIS) in patients with Crohn's disease (CD).

METHODS

This was a retrospective study of 104 patients with CD that were treated at the Ruijin Hospital between March 2015 and May 2016. Among them, 61 patients with active CD were evaluated before/after treatment. MRI and endoscopy were performed within 7 d. CDEIS was evaluated. MRI parameters included MaRIA scores, total relative contrast enhancement (tRCE), arterial RCE (aRCE), portal RCE (pRCE), delay phase RCE (dRCE), and apparent diffusion coefficient. The correlation and concordance between multiple MRI findings and CDEIS changes before and after CD treatment were examined.

RESULTS

Among the 104 patients, 61 patients were classified as active CD and 43 patients as inactive CD. Gender, age, disease duration, and disease location were not significantly different between the two groups (all $P > 0.05$). CRP levels were higher in the active group than in the inactive group (25.12 ± 4.12 *vs* 5.14 ± 0.98 mg/L, $P < 0.001$). Before treatment, the correlations between

CDEIS and MaRIAs in all patients were r = 0.772 for tRCE, r = 0.754 for aRCE, r = 0.738 for pRCE, and r = 0.712 for dRCE (all MaRIAs, P < 0.001), followed by MRI single indexes. Among the active CD patients, 44 cases were remitted to inactive CD after treatment. The correlations between CDEIS and MaRIAs were r = 0.712 for aRCE, r = 0.705 for tRCE, r = 0.685 for pRCE, and r = 0.634 for dRCE (all MaRIAs, P < 0.001).

CONCLUSION

Arterial MaRIA should be an indicator for CD follow-up and dynamic assessment. CD treatment assessment was not completely concordant between CDEIS and MRI.

Key words: Magnetic resonance imaging; Bowel; Crohn's disease; Crohn's disease endoscopic index of severity; Concordance

Core tip: Magnetic resonance imaging (MRI) is accurate in evaluating Crohn's disease (CD) activity and treatment efficacy, but endoscopy (CD endoscopic index of severity) is still the first choice. There are few available data about the concordance between MRI and endoscopy findings before and after treatment. This study provides evidence that MRI indicators are the most sensitive when the disease progresses.

Zhu NY, Zhao XS, Miao F. Magnetic resonance imaging and Crohn's disease endoscopic index of severity: Correlations and concordance. *World J Gastroenterol* 2018; 24(21): 2279-2290 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i21/2279.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i21.2279

INTRODUCTION

Crohn's disease (CD) is an inflammatory bowel disease that may involve the entire gastrointestinal tract[1]. The morbidity of CD has increased in recent years[2]. CD is characterized by segmental and transmural inflammation with nearly 70% involvement of the small bowel, particularly the terminal ileum[3,4]. Since CD can easily recur, accurate and comprehensive evaluation and follow-up are essential to design an individualized treatment program[5].

Magnetic resonance imaging (MRI) of bowels can not only display eventual lesions in the bowel mucosa and sub-mucosa, but also show mesenteric vessel changes and complications. MRI is an important method in the non-invasive diagnosis of CD[6-10]. The Crohn's Disease Endoscopic Index of Severity (CDEIS)[11] is calculated based on endoscopy findings and can only show mucosal ulcers and stenosis. It is generally used to assess CD activity and the accuracy of MRI indicators. Nevertheless, there are few available data about the strength of the association between MRI and CDEIS for the evaluation of CD before and after treatment.

It is currently uncertain whether MRI abnormalities are concordant with changes in CDEIS and whether MRI is only a supplementary/accessory assessment method to endoscopy or could be substituted to endoscopy during follow-up. Of course, MRI is a non-invasive examination, improving the patients' quality of life and compliance to follow-up. Tielbeek *et al*[12] showed that MRI is fairly reproducible but had a moderate agreement with CDEIS; nevertheless, they did not examine the two examinations during follow-up or before/after treatment. Similar results were observed by Rimola *et al*[6,13], but again without follow-up or treatment efficacy assessment.

Therefore, the aim of the present study was to examine the correlation and concordance between multiple MRI findings and CDEIS changes before and after CD treatment. The results could improve our understanding of CD and provide non-invasive modalities for examining the efficacy of treatments.

MATERIALS AND METHODS

Study design and patients

This was a retrospective study of 104 patients with CD and treated at the Ruijin Hospital between March 2015 and May 2016. The project was approved by the ethics committee of the Ruijin Hospital. The need for individual consent was waived because of the retrospective nature of the study.

All patients diagnosed with CD during the study period were included. The exclusion criteria were: (1) Poor MR image quality that could not be used for diagnosis and measurements; or (2) incomplete clinical data. The diagnosis of CD was based on the criteria from the World Health Organization (WHO)[14]. These criteria are: (1) Non-contiguous/segmental lesions visible by imaging, endoscopy, and/or the resected specimen; (2) manifesting as paving stones/longitudinal ulcer visible by imaging, endoscopy, and/or the resected specimen; (3) inflammatory lesions of the entire wall based on clinical manifestations and/or resected specimen showing abdominal masses, and stenosis visible by imaging and endoscopy; (4) histopathological manifestations of non-cheese-like granuloma; (5) cleft/fistula visible by imaging, endoscopy, and/or the resected specimen; and (6) anal lesions visible by clinical manifestations and/or biopsy/resected specimen. The diagnosis of CD is made in the presence of: (1) Criteria 1+2+3 and any one of 4, 5, or 6; or (2) criterion 4 and any two of 1, 2, or 3[14].

Endoscopic and MRI examinations were performed within 7 days. The disease course ranged from 1 to 5 years in all patients.

A first MRI and endoscopy were performed in the 104 included patients. According to the CDEIS score[11] before treatment, the patients were classified as active CD (CDEIS > 6) or inactive CD (CDEIS ≤ 6). A second

MRI and endoscopy were conducted in 61 active CD patients after 24-26 wk of medical therapy with glucocorticoids, infliximab (IFX), or adalimumab (ADA).

Endoscopic examination

Intestinal preparation was performed routinely the night before endoscopy. Double balloon enteroscopy was performed using an oral intubation depth of about 220 cm and a mean anal intubation depth of 120 cm. Colonoscopy was performed by pushing the endoscope from the anus to the distal ileum. All endoscopic examinations were performed by the same two gastroenterologists.

CDEIS

CDEIS was determined as previously reported[11]: CDEIS = (12 × the number of bowel segments with deep ulcers + 6 × the number of bowel segments with superficial ulcers + affected bowel surfaces with no ulcer + ulcerated surface) ÷ the total number of affected segments + 3 × the number of ulcerated stenosis + 3 × the number of stenosis with no ulcer.

MRI

All patients were instructed to fast overnight prior to the MRI examination. The patients were requested to take polyethylene glycol electrolyte powder at 8 PM the day before MRI. Isotonic mannitol solution (2.5%; 2000 mL) was prepared by adding 250 mL of hyperosmotic mannitol solution (0.05 kg of mannitol, concentration of 20%) to 1750 mL of water. Each patient was given three to four 500-mL glasses of isotonic mannitol solution (total, 1500-2000 mL) to optimize the distention of the small bowel. Each glass was given within 10 min. The first glass was given at 40-45 min before MRI. All patients completed bowel preparation before MRI.

All MRI examinations were performed using a 1.5 T MRI unit (GE Signa, HDxt, GE Healthcare, Waukesha, WI, United States). Patients were placed in the supine position with an abdomen coil. MRI was performed with the following sequences: (1) Transverse fast imaging employing steady-state acquisition (FIESTA): Echo time/repetition time (TE/TR) 1.34/3.559 ms, slice thick/gap 5/1 mm, flip angle 55, bandwidth 125, number of excitation (NEX) 1.0, frequency (Freq) 224, field of view (FOV) 40 × 40 cm; (2) coronal T2 Weight Single-Shot Fast Spin Echo (T2WSSFSE): TE/TR 74.56/1800 ms, slice thick/gap 5/1 mm, bandwidth 31.25, Freq 288, FOV 40 cm × 40 cm; (3) coronal FIESTA: TE/TR 1.364/3.285 ms, slice thick/gap 5/1 mm, flip angle 55, bandwidth 125; (4) transverse diffusion weight imaging (DWI): b values were 0, 600 s/mm^2, TE/TR 67.5/1800 ms, slice thick/gap 5/1 mm, Freq 128, NEX 2.0; and (5) coronal Liver Acquisition with Volume Acceleration (LAVA) dynamic enhanced scan: TE/TR 1.452/3.12 ms, slice thickness/gap 4-4.4/1 mm, flip angle 12, bandwidth 125, Freq 288, FOV 40 cm × 40 cm; contrast agent, Magnevist 0.2 mL/kg, injection rate of 2 mL/s, enhanced scan point of 20, 50, and 90 s after contrast agent injection.

All MR images were independently reviewed by two experienced gastrointestinal radiologists who were blinded to the CDEIS results. Since the CDEIS represents the worst segment seen during endoscopy, the radiologists selected the worst segment on MRI for analysis. In the present study, each lesion observed during MRI could be matched to the endoscopy findings.

T2WI can show the intestinal wall thickening, serosal edema (T2WI high signal), and mucosal defects suggesting ulcers[6-11,15]. For each individual, bowel thickness was measured using the T2WI sequence. Wall edema[6-11,15] (hyperintensity on T2WI of bowel wall relative to the signal of the psoas muscle), ulcer in mucosa[6] (deep depression in the mucosal surface of a thickened segment), and reactive lymph nodes (enlarged > 1 cm) were observed in T2WI. LAVA dynamic enhanced sequence was used to evaluate[4,16]: (1) Wall enhancement pattern: layer stratified enhancement or non-layer stratified enhancement; (2) changes in morphology including shortened mesenteric border, pseudodiverticulum, and stenosis; and (3) perienteric exudation, wall edema, ulcer in mucosa, reactive lymph nodes, perienteric exudation, morphological changes, and layer stratified enhancement, each defined as present or absent.

For patients in the active phase, regions of interest (ROIs) of < 0.5-cm^2 were placed on the mucous layer of the lesion segment. In active CD, the mucous layer can be seen clearly due to edema in the sub-mucous layer. For inactive CD, the ROI was placed on the whole bowel wall since the mucous and sub-mucous layers cannot be differentiated. According to a study by Semelka *et al*[17], quantitative measurement of ROIs of wall signal intensity (WSI) was conducted before and after intravenous contrast administration. Relative contrast enhancement (RCE) was calculated according to: $RCE = (WSI_{post\text{-}enhancement} - WSI_{pre\text{-}enhancement})/(WSI_{pre\text{-}enhancement}) \times 100 \times SD_{noisepre\text{-}enhancement}/SD_{noise\ post\text{-}enhancement}$), where $SD_{noise\ pre\text{-}enhancement}$ is the average of three standard deviations (SDs) of the signal intensity measured outside of the body before enhancement, and $SD_{noise\ post\text{-}enhancement}$ presents the same noise after enhancement.

DWI can be used to measure the movement of water molecules in living bodies. In the presence of acute inflammation, the edema, exudation of intestinal wall tissue, and elevated inflammatory cytokine levels limit the movement of the water molecules in tissues and cells (*i.e.,* the diffusion is limited). Hence, the DWI signals increase while apparent diffusion coefficient (ADC) values decrease. Those values are reversed when inflammation improves[6-11,15]. In DWI sequences, ROIs of ADC placed on the bowel wall of CD lesions were measured using the Functool Software, and the average values were obtained. A simplified Magnetic Resonance Index of Activity (MaRIA) was calculated for each segment using the formula 1.5 × wall thickness (mm) + 0.02 × RCE + 5 × edema + 10 × ulceration.

Artery enhancement sequence on T1W1 shows the blood supply of the intestine. aRCE is the enhancement rate during arterial phase and represents the degree of blood supply. pRCE is the blood supply during the portal phase. dRCE is the blood supply during the period of delay. In the presence of acute inflammation, the enhancement rates of the various phases are elevated. If the peak value of the enhancement curve is delayed, the inflammation is likely to be improved or chronic[6-11,15]. The average RCE (total RCE, tRCE; arterial phase RCE, aRCE; portal phase RCE, pRCE; delay phase RCE, dRCE) and ADC values of the lesions in each patient were obtained. ΔtRCE, ΔaRCE, ΔpRCE, ΔdRCE, ΔADC, ΔMaRIA, Δthickness, and ΔCDEIS were defined as Δ CDEIS = (indicators after treatment-indicators before treatment)/indicators before treatment.

If the lesions were improved after medical treatment of CD, the following MRI manifestations could be seen: (1) T2WI showed that the thickening of the intestinal wall was alleviated, edema was alleviated or had disappeared, and mucosal ulcers were healed; (2) dynamic T1W1 enhancement sequence showed that the enhancement of the lesion segment had weakened, and the intestinal wall was no longer stratified; (3) the exudation surrounding the intestines was reduced or had disappeared, and the enlarged lymph nodes surrounding the intestines had shrunk; and (4) DWI sequence showed that the signals of the diseased segment were reduced and ADC values were increased.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 for Windows (IBM, Armonk, NY, United States). Categorical variables (intestinal wall edema, ulcer in mucosa, reactive lymph nodes, perienteric exudation, wall enhancement pattern, and morphological changes) were analyzed using the Spearman correlation. Continuous variables (bowel thickness, tRCE, aRCE, pRCE, dRCE, ADC values, MaRIA) were expressed as mean ± standard deviation, and the Pearson correlation analysis was performed. ΔMRI indicators were analyzed with ΔCDEIS using the Pearson correlation. An inter-observer agreement evaluation between the two radiologists was performed using the kappa statistics. Two-sided *P*-values < 0.05 were considered statistically significant.

RESULTS

Characteristics of the patients

Table 1 presents the characteristics of the patients. Among the 104 patients, 61 patients (male/female, 36/25; mean age, 27.5 ± 11.4 years) were classified as having active CD (CDEIS > 6) and 43 patients (male/female, 24/19; mean age, 24.4 ± 8.0 years) as having inactive CD (CDEIS ≤ 6). Gender, age, disease duration, and disease location were not significantly different between the two groups (all $P > 0.05$). C-reactive protein (CRP) levels were higher in the active group than in the inactive group ($P < 0.001$).

MRI agreement

An inter-observer agreement evaluation between the two radiologists was performed using kappa statistics, which showed a high correlation (0.936) when considering all parameters. Therefore, the average results of the two radiologists were used for evaluation.

Before treatment

Table 1 presents the MRI findings before treatments. Higher proportions of patients in the active group showed edema, mucosal ulcer, enhancement pattern, morphological changes, and perienteric exudation than in the inactive group (all $P < 0.05$).

On MRI and compared with the inactive group, the active group showed lower ADC ($P = 0.001$) and higher thickness, tRCE, aRCE, pRCE, dRCE, and MaRIA (all $P < 0.05$). At endoscopy, the active group showed higher CDEIS scores than the inactive group ($P = 0.001$) (Table 1).

MRI quantitative parameters (ADC value, bowel thickness, tRCE, aRCE, pRCE, dRCE, and MaRIA scores) were significantly correlated with CDEIS. The highest correlation was found between MaRIA and CDEIS with coefficients of $r = 0.772$ for tRCE, $r = 0.754$ for aRCE, $r = 0.738$ for pRCE, and $r = 0.712$ for dRCE, followed by tRCE, aRCE, pRCE, dRCE, bowel thickness, and ADC value ($r = 0.661$, 0.634, 0.518, 0.507, 0.356, and -0.276, respectively) (Table 1).

In the active CD group, CDEIS was significantly correlated with MaRIAs, tRCE, aRCE, pRCE, dRCE, bowel thickness, and ADC, with coefficients of $r = 0.789$, 0.767, 0.745, 0.718, 0.726, 0.548, 0.54, 0.459, 0.311, and -0.207, respectively (Table 2). On the other hand, in the inactive CD group, MaRIA (for tRCE), and tRCE were positively correlated with CDEIS ($r = 0.746$ and 0.718, respectively) (Table 2, and Figures 1 and 2).

After treatment

All 61 patients in the active group underwent MRI and endoscopy examinations after medical treatment. The correlation coefficients between CDEIS and MaRIAs were $r = 0.771$ for MaRIA of aRCE, $r = 0.755$ for MaRIA of dRCE, $r = 0.740$ for MaRIA of pRCE, and $r = 0.736$ for MaRIA of tRCE, which were all higher than that between CDEIS and single MRI parameters. Among single MRI indicators, the highest correlation was found for aRCE. The same correlation order was found between ΔMaRIAs and ΔCDEIS as that between MaRIAs and CDEIS. For single ΔMRI indicators, the correlation was in the order of ΔaRCE > ΔADC > ΔpRCE > ΔdRCE > ΔtRCE, with $r = 0.593$, -0.545, 0.529, 0.512, and 0.467, respectively (Table 3). No correlation was observed between CDEIS and bowel thickness (Table 3).

After treatment, 17 of the 61 patients remained with active CD. Table 4 presents the characteristics of these patients. Gender, age, disease duration, disease

Table 1 Characteristics of the patients before treatments

	All n = 104	Active n = 61	Inactive n = 43	P value
Gender (M/F)	59/45	36/25	23/20	0.230
Age	31.37 ± 9.56	27.5 ± 11.4	34.44 ± 5.37	0.650
Disease duration	3.5	3.9	3.3	0.550
Disease location				
Rectum	0	0	0	
Sigmoid/left colon	4	2	2	
Transverse colon	14	7	7	
Right colon	16	10	6	
Ileum	70	42	28	
Treatment regimen				
Glucocorticoid	23	23	0	
IFX	18	18	0	
ADA	20	20	0	
Edema	61	61	0	< 0.001
Reactive lymph nodes	25	16	9	0.311
Mucosal ulcer	49	38	11	< 0.001
Enhancement pattern	61	61	0	0.006
Morphological changes	31	12	19	0.023
Perienteric exudation	38	38	0	< 0.001
CRP (mg/L)	18.34 ± 8.45	25.12 ± 4.12	5.14 ± 0.98	< 0.001
ADC (mm^2/s)	1.87 ± 0.471	1.598 ± 0.383	1.949 ± 0.431	0.001
Thickness (mm)	7.89 ± 3.23	9.23 ± 3.36	6.75 ± 2.49	0.001
tRCE (%)	78.34 ± 45.34	92.153 ± 101.34	40.592 ± 11.019	0.017
aRCE (%)	124.45 ± 61.11	181.46 ± 97.80	92.63 ± 45.48	< 0.001
pRCE (%)	254.21 ± 198.22	321.90 ± 231.03	201.32 ± 124.66	0.020
dRCE (%)	377.15 ± 223.21	466.18 ± 260.08	271.91 ± 209.66	0.002
MaRIA				
tRCE	20.37 ± 3.42	26.18 ± 5.02	6.44 ± 1.03	< 0.001
aRCE	18.88 ± 4.11	28.40 ± 4.84	6.43 ± 2.74	< 0.001
pRCE	26.32 ± 2.89	35.09 ± 4.64	6.94 ± 2.58	< 0.001
dRCE	19.26 ± 3.21	36.81 ± 5.11	7.25 ± 2.32	0.001
CDEIS	8.15 ± 4.03	10.57 ± 3.02	3.46 ± 1.23	0.001

IFX: Infliximab; ADA: Adalimumab; CRP: C-reactive protein; ADC: Apparent diffusion coefficient; tRCE: Total relative contrast enhancement; aRCE: Arterial relative contrast enhancement; pRCE: Portal phase relative contrast enhancement; dRCE: Delay phase relative contrast enhancement; MaRIA: Magnetic resonance index of activity; CDEIS: Crohn's disease endoscopic index of severity.

Table 2 Correlations between magnetic resonance indicators and Crohn's disease endoscopic index of severity before treatment in the two groups

	All n = 104		Active group n = 61		Inactive group n = 43	
	r	P value	r	P value	r	P value
ADC	-0.276	0.012	-0.207	0.016	-0.202	0.356
Thickness	0.356	0.001	0.311	0.002	0.952	0.013
tRCE	0.661	< 0.001	0.726	< 0.001	0.718	< 0.001
aRCE	0.634	< 0.001	0.548	< 0.001	0.238	0.274
pRCE	0.519	< 0.001	0.540	< 0.001	0.921	0.022
dRCE	0.507	< 0.001	0.459	< 0.001	0.022	0.920
MaRIA						
tRCE	0.772	< 0.001	0.789	< 0.001	0.746	< 0.001
aRCE	0.754	< 0.001	0.767	< 0.001	0.334	0.288
pRCE	0.738	< 0.001	0.745	< 0.001	0.230	0.471
dRCE	0.712	< 0.001	0.718	< 0.001	0.280	0.378
CDEIS	8.15 ± 4.03		10.57 ± 3.02		3.46 ± 1.23	

ADC: Apparent diffusion coefficient; tRCE: Total relative contrast enhancement; aRCE: Arterial relative contrast enhancement; pRCE: Portal phase relative contrast enhancement; dRCE: Delay phase relative contrast enhancement; MaRIA: Magnetic resonance index of activity; CDEIS: Crohn's disease endoscopic index of severity.

location, and CRP levels were similar between the two groups. The inactive group showed better clinical and MRI performances than the active group after treatments (all P < 0.05). In those 17 patients, no statistical correlation was found between endoscopy score and MRI indicators. The remaining 44 patients remitted into inactive CD. The correlations between CDEIS and MRI parameters in these 44 cases were in the order of MaRIA for aRCE > MaRIA for tRCE > MaRIA for pRCE > MaRIA for dRCE > aRCE > ADC

Table 3 Correlations between magnetic resonance imaging indicators and Crohn's disease endoscopic index of severity in the 61 active Crohn's disease patients after treatment

CDEIS			ΔCDEIS		
	r	*P* value		*r*	*P* value
ADC	-0.467	< 0.001	ΔADC	-0.545	0.001
Thickness	0.242	0.201	Δthickness	0.407	0.148
tRCE	0.548	0.002	ΔtRCE	0.467	0.018
aRCE	0.619	< 0.001	ΔaRCE	0.593	0.002
pRCE	0.493	0.008	ΔpRCE	0.529	0.004
dRCE	0.490	0.015	ΔdRCE	0.512	0.003
MaRIA			ΔMaRIA		
tRCE	0.736	< 0.001	tRCE	0.724	< 0.001
aRCE	0.771	< 0.001	aRCE	0.781	< 0.001
pRCE	0.740	< 0.001	pRCE	0.724	< 0.001
dRCE	0.755	< 0.001	dRCE	0.760	< 0.001

ADC: Apparent diffusion coefficient; tRCE: Total relative contrast enhancement; aRCE: Arterial relative contrast enhancement; pRCE: Portal phase relative contrast enhancement; dRCE: Delay phase relative contrast enhancement; MaRIA: Magnetic resonance index of activity; CDEIS: Crohn's disease endoscopic index of severity.

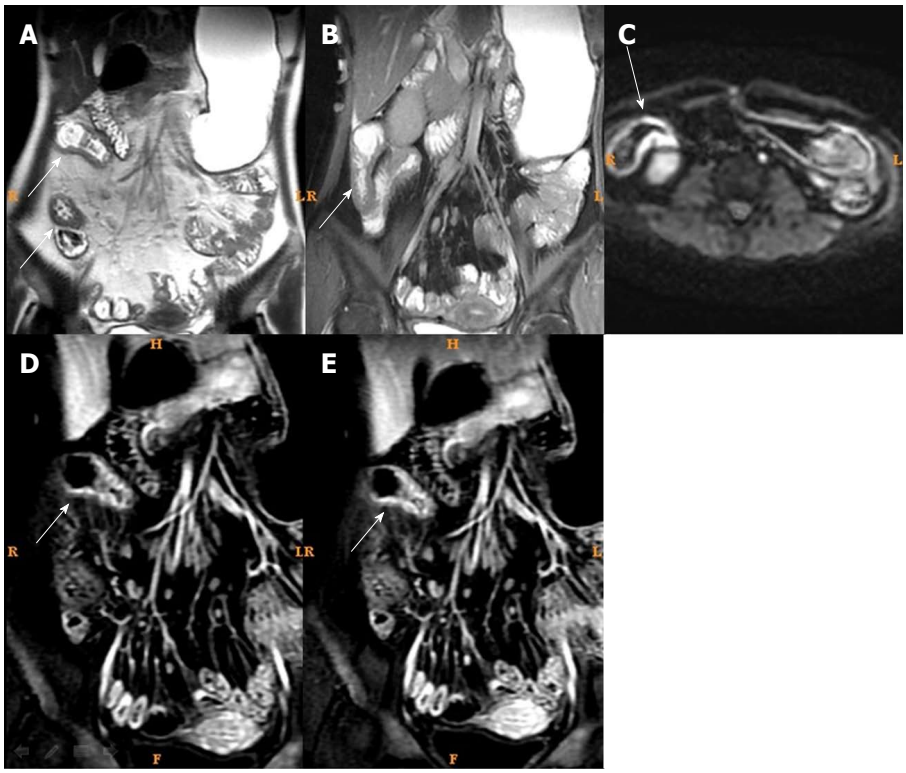

Figure 1 Magnetic resonance imaging of a typical case of active Crohn's disease before treatment. Female, 32 years of age, active Crohn's disease. A: T2WI showed intestinal wall thickening and submucosal edema in the distal ileum; B: Fast imaging employing steady-state acquisition showed intestinal wall thickening and submucosal edema in the distal ileum; C: Diffusion weight imaging showed marked high intensity; D and E: Dynamic enhancement showed obvious layer stratified enhancement.

value > tRCE > pRCE > dRCE, with *r* = 0,712, 0.705, 0.685, 0.634, 0.697, -0.516, 0.420, 0.350, and 0.341, respectively (Table 5).

Among MRI qualitative indicators, statistical analysis could not be done for mucosal ulcer because of its low frequency (16/61). Edema in the submucosa and perienteric exudation were decreased (61/61 and 18/18) after treatment. In addition, the enhancement pattern of the bowel wall in inactive CD patients changed to non-stratified enhancement (44/61), whereas it remained stratified enhancement in active CD patients (17/61) (Figures 3 and 4).

DISCUSSION

MRI is fairly reproducible but shows only a moderate agreement with CDEIS[6,12,13]. Furthermore, the concordance of the two examinations during follow-up or before/after treatment remains uncertain. Therefore, this study aimed to examine the correlation and concordance

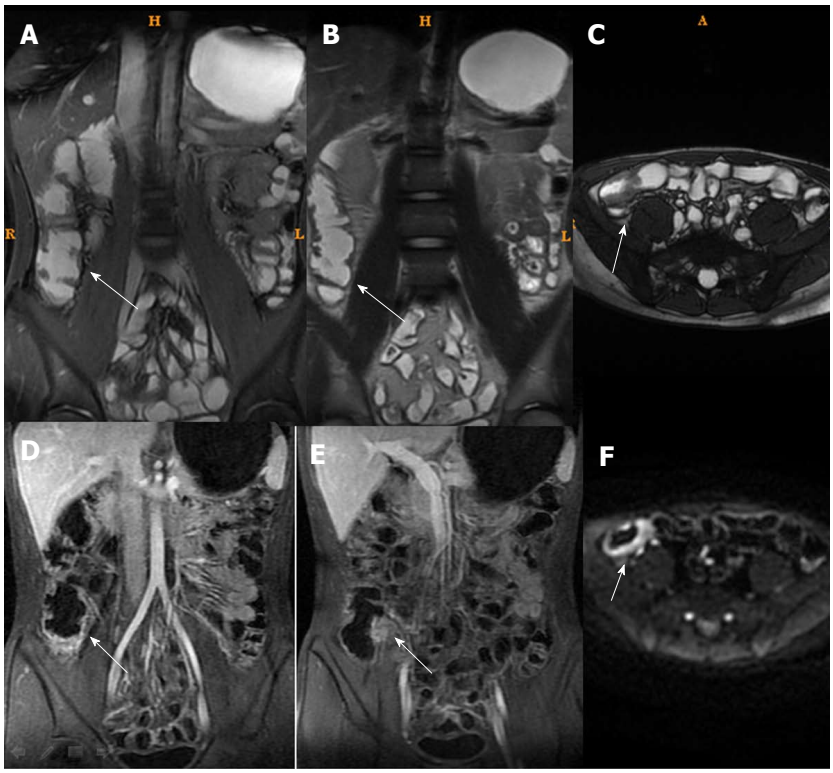

Figure 2 Magnetic resonance imaging of a typical case of active Crohn's disease after treatment (same patient as in Figure 1). She remained in the active Crohn's disease group after treatment. A: T2WI showed intestinal wall thickened and submucosal edema decrease in the distal ileum; B: Fast imaging employing steady-state acquisition showed intestinal wall thickened and submucosal edema decrease in the distal ileum; C: Diffusion weight imaging showed less high intensity; D and E: Dynamic enhancement showed layer stratified enhancement.

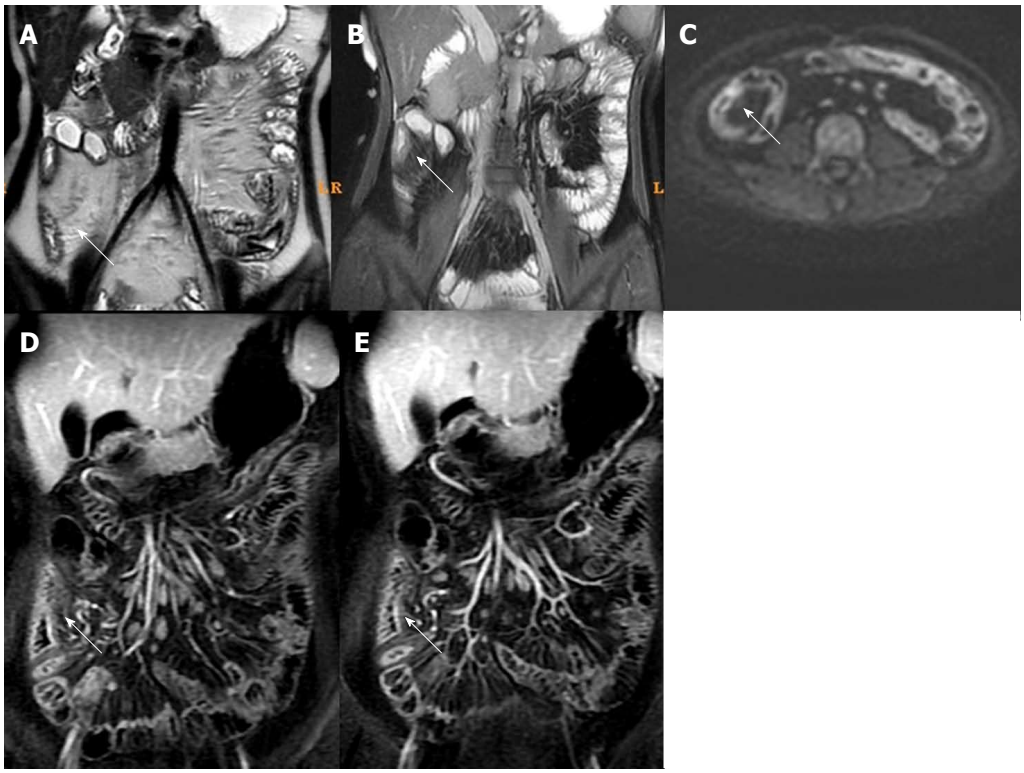

Figure 3 Magnetic resonance imaging of a typical case of active Crohn's disease before treatment. Male, 25 years of age, active Crohn's disease. A: Fast imaging employing steady-state acquisition showed intestinal wall thickening and submucosal edema in the ascending colon; B and C: T2WI showed intestinal wall thickening and submucosal edema in the ascending colon; D and E: Dynamic enhancement showed obvious enhancement; F: Diffusion weight imaging showed marked high intensity.

Table 4 Subgroups in the active Crohn's disease patients according to disease activity after treatments

	Remained active (n = 17)		Improved to inactive (n = 44)		P value
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	
Gender (M/F)	10/7		26/18		0.332
Age	30.4 ± 5.5		26.7 ± 10.1		0.563
Disease duration	1-5		1-5		
Disease location					0.916
Sigmoid/left colon	1		1		
Transverse colon	3		4		
Right colon	4		6		
Ileum	14		28		
Treatment regimen					0.292
Glucocorticoids	8		15		
Infliximab	6		12		
Adalimumab	3		17		
CRP	28.01 ± 5.22		20.91 ± 5.45		0.054
CDEIS	12.23 ± 5.12	10.47 ± 3.43	10.12 ± 2.11	3.11 ± 0.21	0.001
CRP	28.01 ± 5.215	15.12 ± 4.32	20.91 ± 5.45	5.84 ± 0.743	0.002
ADC	1.52 ± 0.12	1.44 ± 0.34	1.59 ± 0.17	1.73 ± 0.2	0.001
Thickness	9.12 ± 1.21	7.66 ± 1.41	8.2 ± 2.22	5.42 ± 1.32	0.012
tRCE	89.14 ± 13.33	69.49 ± 12.11	82.11 ± 12.47	45.32 ± 4.53	0.021
aRCE	179.03 ± 20.66	166.16 ± 22.44	181.14 ± 34.1	89.76 ± 12.71	0.001
pRCE	330.02 ± 67.12	285.27 ± 57.71	301.32 ± 54.12	199.23 ± 23.2	0.001
dRCE	453.29 ± 54.05	385.5 ± 45.32	440.18 ± 33.09	257.22 ± 44.13	0.001
MaRIA					
tREC	35.17 ± 5.66	30.12 ± 3.12	26.56 ± 2.90	6.23 ± 1.11	< 0.001
aRCE	28.22 ± 6.76	19.12 ± 4.09	29.47 ± 5.22	6.48 ± 1.38	< 0.001
pRCE	37.79 ± 5.59	29.21 ± 4.21	36.28 ± 4.72	7.11 ± 1.74	< 0.001
dRCE	36.09 ± 8.12	25.2 ± 5.77	27.08 ± 5.79	7.22 ± 1.59	< 0.001
ESR	24.186 ± 3.210	18.28 ± 3.38	21.49 ± 3.33	3.184 ± 0.568	< 0.001

CRP: C-reactive protein; ADC: Apparent diffusion coefficient; tRCE: Total relative contrast enhancement; aRCE: Arterial relative contrast enhancement; pRCE: Portal phase relative contrast enhancement; dRCE: Delay phase relative contrast enhancement; MaRIA: Magnetic resonance index of activity; CDEIS: Crohn's disease endoscopic index of severity; ESR: Erythrocyte sedimentation rate.

Table 5 Correlations between magnetic resonance indicators and Crohn's disease endoscopic index of severity in the active group according to disease activity after treatment

	Remained active n = 17		Improved to inactive n = 44	
	r	P value	r	P value
ADC	-0.219	0.518	-0.516	0.002
Thickness	0.105	0.758	0.170	0.568
tRCE	0.356	0.203	0.420	0.005
aRCE	0.376	0.255	0.697	0.002
pRCE	0.305	0.113	0.350	0.010
dRCE	0.381	0.134	0.341	0.015
MaRIA				
tRCE	0.268	0.400	0.705	< 0.001
aRCE	0.268	0.399	0.712	< 0.001
pRCE	0.306	0.334	0.685	< 0.001
dRCE	0.309	0.329	0.634	< 0.001

ADC: Apparent diffusion coefficient; tRCE: Total relative contrast enhancement; aRCE: Arterial relative contrast enhancement; pRCE: Portal phase relative contrast enhancement; dRCE: Delay phase relative contrast enhancement; MaRIA: Magnetic resonance index of activity.

among multiple MRI findings and CDEIS changes before and after CD treatment. The results strongly suggest that MRI artery phase-enhanced indexes were the most sensitive indicators, especially arterial MaRIA, for CD follow-up and dynamic assessment of the therapeutic effects. CD treatment assessment was not completely concordant between CDEIS and MRI.

The clinical course of CD usually presents an acute-remission-recur cycle. Therefore, regular monitoring and follow-up are needed. The assessment methods for the diagnosis and follow-up include clinical manifestations, endoscopy, histopathology, computed tomography (CT), and MRI[18]. In clinical practice, there is often a low correlation between clinical symptoms and bowel inflammatory activity. Clinical symptoms may be unrelated to endoscopy and imaging findings[19,20]. Endoscopy and histopathology exams are the first choice for the diagnosis of CD[1,18]. Nevertheless, these approaches are invasive

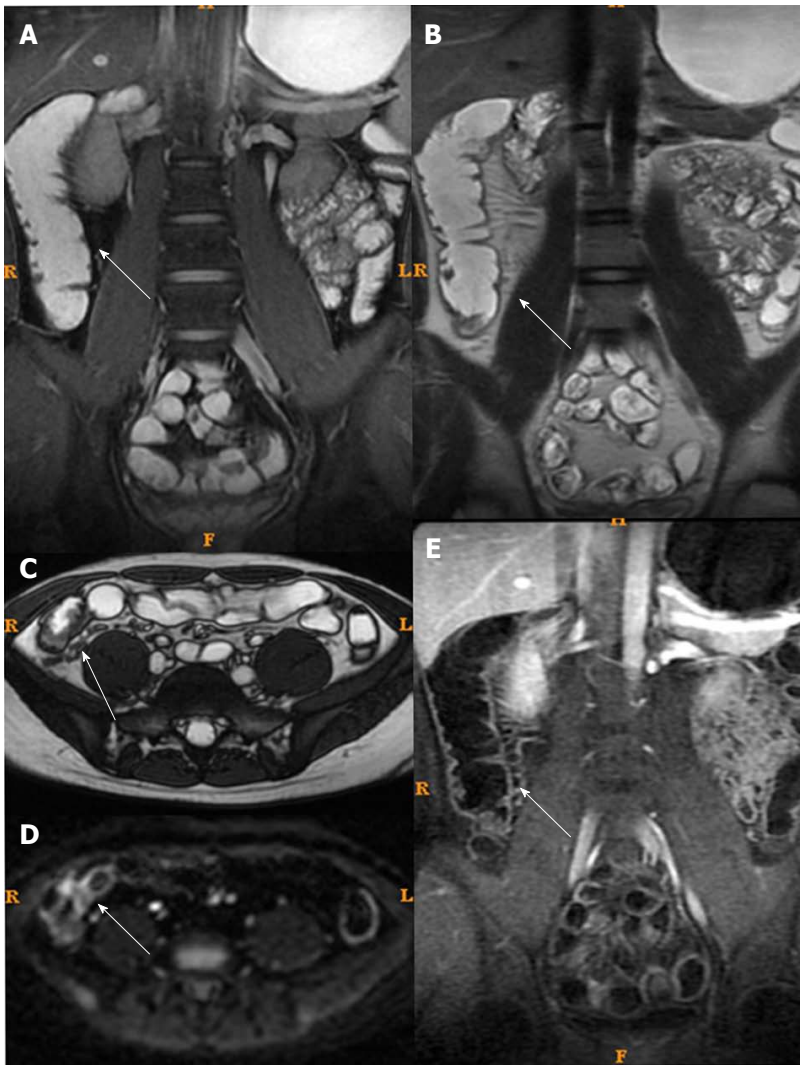

Figure 4 Magnetic resonance imaging of a typical case of active Crohn's disease after treatment (same patient as in Figure 3). The patient was in remission (inactive Crohn's disease) after treatment. A: Fast imaging employing steady-state acquisition showed decreased intestinal wall thickening and no submucosal edema; B and C: T2WI showed decreased intestinal wall thickening and no submucosal edema; D: Diffusion weight imaging showed less high intensity; E: Enhancement showed less enhancement.

and ill-suited for regular monitoring and follow-up. Therefore, MRI is probably one of the most appropriate methods for the long-term evaluation and monitoring of CD.

An early study on the efficacy of CD treatment was reported using MRI enhanced index and bowel thickness[21]. Other MRI evaluations, such as mucosal ulcer and ADC value, were used in recent studies[22]. Some studies[23-27] focused on the accuracy of MRI indicators for the evaluation of CD and the response to medical therapy. One study reported that changes in CD clinical activity were significantly correlated with changes in MRI activity score[28]. Bowel wall thickening, mesenteric lymphadenopathy, and fat wrapping with vascular proliferation were the MRI parameters that changed significantly after induction and maintenance treatment in responders[28]. The changes in MRI activity score were mostly pronounced during the first 3 months of treatment compared with long-term treatments (weeks 52-54)[28]. In the present study, both MRI scores (MaRIA) and single MRI indicators (ADC, tRCE, aRCE, pRCE, dRCE, and bowel thickness) were evaluated. After treatment, MaRIA scores, ADC, tRCE, aRCE, pRCE, dRCE, ΔADC, ΔtRCE, ΔaRCE, ΔpRCE, and ΔdRCE remained correlated with CDEIS, but bowel thickness was not, possibly because CD is a chronic and recurrent disease. Both edema and chronic fibrosis can be found in thickened bowel segment. After effective medical therapy, inflammation may be improved and edema may have regressed, but fibrous adipose tissue hyperplasia may be present or become more serious. This may weaken the correlation between bowel thickness and CDEIS. Secondly, compared with other studies, the evaluation timing after treatment was different. Therefore, the decision when to make the MRI evaluation is still an issue. Various MRI assessment timings may produce different results in treatment effect. Thirdly, our sample size was limited. Nevertheless, a recent study supports the use of MaRIA for the evaluation of CD[29].

In the present study, higher correlations were found

for MaRIA scores than that for single MRI indicators. Among them, the MaRIA score of aRCE showed the best correlation after treatment. Among single MRI indicators, the best correlation was found between aRCE and CDEIS. Possible reasons that MRI artery phase enhanced indexes were the most sensitive for efficacy assessment after CD treatment may be decreased blood supply to mucosal ulcer and improved inflammation.

Nevertheless, each correlation coefficient of enhanced indexes was decreased compared with those before treatment in 61 patients with CD. In general, MRI findings, as a treatment evaluation method, were not completely matched with CDEIS, especially after 24-26 wk of effective treatment. Grouped by treatment effect, good correlation between MRI and CDEIS results was found in active CD patients who experienced remission but not among those who remained with active CD after treatment. This finding may also reflect that time has an impact on the changes between MRI and CDEIS.

DWI has recently been shown to be an appropriate tool for the follow-up of CD[30,31]. In the present study, the correlation between ADC values and CDEIS after treatment was increased compared with that before treatment, especially ΔADC. Though ADC value was proved to be a reliable independent indicator for the evaluation of CD and with a similar value to that of enhancement indicators in previous studies[9,22], the present study showed that it was more valuable and reliable to follow-up the change of ADC values for dynamic monitoring. It had a good value reflecting CD prognosis during periods rather than at specific time point of the disease.

Among qualitative indicators, because of strict requirement for bowel distension, no advantage was shown for MRI detecting mucosal ulcer compared with endoscopy. Other MRI indicators, such as edema, exudation, and enhancement pattern, were sensitive and matched the CDEIS changes before and after treatment. Because these are subjective indicators and may vary among observers, they seem to be less accurate and dependable indicators compared with RCE and ADC values. Nevertheless, a study showed that endoscopy and MRI were concordant, even without bowel preparation[32]. Additional studies are warranted on this point.

The present study is not without limitations. The sample size was small and from a single center. In addition, the retrospective nature of the study prevented the study of parameters that were not routinely collected. Thirdly, all treated patients were grouped together, but different treatments might have different impact on MRI findings. Finally, MRI T2W1 and T1W1 dynamic enhancement sequences can show intestinal fistula but, in the present study, the frequency of fistula was low. Therefore, reliable statistical analyses could not be performed. Additional studies are necessary to improve upon these results.

In conclusion, MRI indicators were correlated with CDEIS, but such correlation was decreased in patients with active CD that became inactive after treatment. CD treatment assessment was not completely concordant between CDEIS and MRI. MRI artery phase enhanced indexes seemed to be the most sensitive indicators, especially MaRIA score of aRCE. MaRIA scores were better than single MRI indicators for CD follow-up and dynamic assessment of therapeutic effects.

ARTICLE HIGHLIGHTS

Research background

Crohn's disease (CD) is an inflammatory bowel disease that may involve the entire gastrointestinal tract. CD easily recurs, and accurate and comprehensive evaluation and follow-up are essential to design an individualized treatment program. Crohn's Disease Endoscopic Index of Severity (CDEIS) is generally used to assess CD activity. However, it is currently uncertain whether MRI abnormalities are concordant with changes in CDEIS. In addition, whether MRI is only a supplementary/accessory assessment method to endoscopy or could substitute endoscopy during follow-up remains unclear.

Research motivation

The clinical symptoms of CD may be unrelated to endoscopy and imaging findings. Endoscopy and histopathology are the first methods of choice for the diagnosis of CD. Nevertheless, these approaches are invasive and ill-suited for regular monitoring and follow-up. Therefore, MRI is probably one of the most appropriate methods for long-term evaluation and monitoring of CD.

Research objectives

We hypothesized that CDEIS changes correlated with MaRIA scores as well as individual MRI parameters before and after CD treatment. The present study aimed to help us to understand the pathological changes of CD and provide non-invasive modalities for examining therapeutic effects.

Research methods

One hundred and four patients with CD were analyzed retrospectively. Among them, 61 and 43 patients were considered to have active CD (CDEIS > 6) and inactive CD (CDEIS ≤ 6), respectively. MaRIA scores as well as individual MRI parameters, including total relative contrast enhancement (tRCE), arterial RCE (aRCE), portal RCE (pRCE), delay phase RCE (dRCE), and apparent diffusion coefficient (ADC), were evaluated. Correlation and concordance between multiple MRI findings and CDEIS were examined.

Research results

In the present study, we found that CDEIS had correlations with MaRIAs at baseline in all patients, including tRCE, aRCE, pRCE, dRCE (all MaRIAs, $P < 0.001$), followed by single MRI indexes. Among the 61 active CD patients, 44 cases were remitted to inactive CD after treatment. In the 44 patients who achieved remission, correlations between CDEIS and all MaRIAs remained after treatment. However, the values of the correlation coefficient (r) were decreased. The most significant correlations were found between MaRIAs for aRCE and CDEIS.

Research conclusions

MRI indicators had correlations with CDEIS in patients with active CD before treatment. However the correlations were decreased in patients with active CD that became inactive after treatment. The assessment was not completely concordant between CDEIS and MRI in patient with CD before and after treatment. The MaRIA score of aRCE seemed to be an important indicator. For dynamic assessment of therapeutic effects, MaRIA scores were better than single MRI indicators.

Research perspectives

Endoscopic results were not completely consistent with MR data among CD patients. The most sensitive indicators in evaluating efficacy by MR

were relevant indicators during the MR enhanced arterial phase. The most appropriate timing for performing MR evaluation and monitoring disease conditions after treatment of CD should be explored in the future.

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ORIGINAL ARTICLE

Clinical Trials Study

Fiber-enriched diet helps to control symptoms and improves esophageal motility in patients with non-erosive gastroesophageal reflux disease

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Institutional review board statement: Study design, protocol, and patients' informed consent form were approved by the Institute of Nutrition IRB (Moscow, Russia), (protocol #12, 25APR2012).

Clinical trial registration statement: This study registered at www.ClinicalTrials.gov, and clinical trial registry number: NCT01882088.

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Abstract

AIM

To investigate the effect of dietary fiber on symptoms and esophageal function testing parameters in non-erosive gastroesophageal reflux disease (GERD) (NERD) patients.

METHODS

Thirty-six NERD patients with low (< 20 g/d) dietary fiber

intake were enrolled in the study. They were examined with the use of symptom questionnaire (GERD-Q), high-resolution esophageal manometry, 24-h esophageal pH-impedance examinations, and food frequency questionnaire before and after 10 d of usual diet supplemented by psyllium 5.0 g TID. Complete data of 30 patients were available to the final analysis. The obtained results were analyzed with the use of non-parametric statistics (Wilcoxon matched pairs test).

RESULTS

The number of patients experiencing heartburn was less (93.3% at baseline *vs* 40% at the end of the study, $P < 0.001$) and the GERD-Q score decreased (mean ± SD: 10.9 ± 1.7 *vs* 6.0 ± 2.3, $P < 0.001$) after the treatment period. Minimal resting lower esophageal sphincter (LES) pressure increased from 5.41 ± 10.1 to 11.3 ± 9.4 mmHg ($P = 0.023$), but no change in residual LES pressure and mean resting pressure was found. Total number of gastroesophageal refluxes (GER) decreased from 67.9 ± 17.7 to 42.4 ± 13.5 ($P < 0.001$) predominantly by acid and weak acid types of GERs. No significant change in mean esophageal pH and % of time pH < 4 was registered. Maximal reflux time decreased from 10.6 ± 12.0 min to 5.3 ± 3.7 min ($P < 0.05$).

CONCLUSION

Fiber-enriched diet led to a significant increase of minimal lower esophageal sphincter resting pressure, a decrease of number of gastroesophageal refluxes, and a decrease of heartburn frequency per week in NERD.

Key words: Gastroesophageal reflux disease; Psyllium; Gastroesophageal reflux; Lower esophageal sphincter relaxation; Esophageal motility; Dietary fiber; Heartburn; Non-erosive gastroesophageal reflux disease

Core tip: Low dietary fiber intake is associated with decreased stomach and gut motility and delayed gastric emptying, which may contribute to the risk of gastroesophageal reflux and gastroesophageal reflux disease (GERD) symptom frequency. The ability of dietary fibers to bind nitric oxide contained in food may diminish its negative effect on lower esophageal sphincter pressure. Our study is the first prospective trial demonstrating that increasing dietary fiber intake results in an increase of minimal esophageal resting pressure, a decrease in the number of gastroesophageal refluxes, and a decrease in heartburn episodes per week in patients with non-erosive GERD.

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INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common condition that is characterized by reflux of gastric content into the esophagus and is associated with symptom-related quality of life decrease and numerous complications[1-4]. Impaired gastroesophageal motility with an increased number of transient lower esophageal sphincter relaxations (TLESR), acidification of the esophagus, and low esophageal clearance are considered to be the most important factors in the pathogenesis of GERD[1,5-7]. Current treatment of GERD includes lifestyle modification, antisecretory drug use, and anti-reflux surgery[6-9]. While healing of reflux esophagitis requires profound suppression of gastric acid secretion and long-term use of maintenance treatment with proton pump inhibitors (PPIs), patients with non-erosive GERD (NERD) may also benefit from other treatment options, like lifestyle or diet modification[10]. Dietary fiber supplementation may be one of the nutrients used for usual diet modification in GERD patients. It was shown that decreased stomach and gut motility, prolonged period of gastric content evacuation, and gastric over-distension associated with low dietary fiber intake and low fiber consumption may play a crucial role in formation of hiatal hernia, which negatively interferes with anti-reflux barrier[11-13]. Increased intragastric pressure and decreased motility are also established risk factors of gastroesophageal reflux[14-22]. The beneficial effect of dietary fiber on esophageal motility in GERD patients is also assumed to be mediated through its ability to bind nitric oxide contained in food and diminish its negative influence on lower esophageal sphincter (LES) pressure[23,24]. It was demonstrated that some of the dietary fibers may affect not only the rate of gastric emptying but also decrease gastric acidity, making the number of gastroesophageal refluxes lower and reducing their damaging capacity[25].

There is no direct evidence to date on the positive influence of dietary fiber on GERD. Therefore, the aim of the present study was to evaluate the effect of dietary fiber on the presence of gastroesophageal reflux, esophageal acidity, lower esophageal sphincter pressure, and clinical manifestations of non-erosive gastroesophageal reflux disease in patients with low dietary fiber intake.

MATERIALS AND METHODS

This single-center open-label prospective study was performed from 2012 to 2016 at the Department of Gastroenterology and Hepatology of Federal Research Center of Nutrition and Biotechnology according to Good Clinical Practice guidelines, the Declaration of Helsinki (1996). Study design, protocol and patients' informed consent form were approved by the Institute of Nutrition IRB (Moscow, Russia). This study was registered on the ClinicalTrials.gov website (NCT01882088).

Enrollment criteria

Enrollment criteria were: willingness to participate in the study (signed informed consent); clinical diagnosis of NERD; dietary fiber deficiency confirmed by validated dietary questionnaire; and pathological gastroesophageal reflux by 24-h esophageal pH-impedance. Exclusion criteria were as follows: antisecretory (PPI, H_2-histamine receptor blockers) drug use 14 d before day 0, concomitant medications including nitrates, beta-adrenergic blocking agents, calcium channels blockers, and any of the hormones (topic steroids for less than 14 d before enrollment were allowed), inability to perform any of the diagnostic procedures required by the study protocol, allergy to psyllium, previous abdominal surgery, and general condition of the patient not allowing to participate in the study by the opinion of the investigator.

Diagnosis of non-erosive form of GERD

Step 1: The presence of GERD symptoms, their severity and frequency were evaluated by certified gastroenterologists. The primary selection criteria for GERD patients was the presence of heartburn and acid regurgitation for at least 2 times a week. These symptoms were verified with a language-specific version of the international GERD-Q questionnaire[26]. Symptom score of 8 points or higher was considered a positive for the presence of GERD. All patients included in the study had a history of heartburn for more than 6 mo and a previous response to acid suppressive therapy (either PPIs or H_2-hystamine receptor blockers). We did our best to exclude other reasons that would mask the disease (*i.e.*, excluded the use of medication known to affect esophageal motility and sensing; excluded functional heartburn, *etc.*).

Step 2: Endoscopy studies were performed using Exera II CV-180 panendoscope (Olympus Ltd, Osaka, Japan). Absence of esophageal erosions and positive results on the GERD-Q questionnaire were necessary to proceed with further examination.

High-resolution esophageal manometry (HRM). HRM studies were performed using a solid-state 36 channel 10Fr catheter (UniTip, Unisensor AG, Portsmouth, NH, United States) inserted transnasally from the pharynx to the stomach after fasting. After the patients were allowed time to adapt to the catheter placement, they were usually given 10 liquid swallows of 5 mL water. Standard software was used to analyze the obtained results (Solar GI, MMS, Enschede, the Netherlands)[27-30]. Mean and minimal resting pressure of lower esophageal sphincter pressure at rest and after 10 swallows of water, residual pressure and percent of relaxation, and their change after the course of treatment were recorded. Any type of achalasia or signs of major motility disorders by Chicago classification v 3.0[30] were exclusion criteria.

Step 3: Twenty-four h esophageal pH-impedance. Twenty-four h esophageal pH-impedance studies were made with the use of Ohmega equipment (MMS, Enschede, the Netherlands) and 2pH-6 impedance channels catheters (UniTip). The studies were performed by the standard technique[31-33]. Catheters were inserted transnasally and located with esophageal pH electrode 5 cm above the upper border of the lower esophageal sphincter, as defined by high resolution manometry. Patients were instructed to press the event marker button on the pH data logger to mark their meal times (then excluded from the analysis), body posture, symptom occurrence, and drug intake. These events together with time of onset were also marked by the patients into the paper diary to exclude mistakes. Patients were encouraged to maintain their normal daily activities throughout the measurement and to continue their regular diet. Manual review of the tracings was performed by experienced operators. Reflux episodes were defined as a decrease from baseline of more than 50% impedance moving from the distal to the proximal extent.

Step 4: Dietary intake of energy and macro- and micronutrients were determined using a validated PC-based Food Frequency Questionnaire (FFQ-1.0, Institute of Nutrition, Moscow, Russia). Dietary fiber intake deficiency was established when daily fiber intake was less than 20 g/d.

If the presence of NERD by endoscopy and GERD-Q questionnaire and low dietary fiber intake were confirmed, eligible subjects were examined with the use of high-resolution esophageal manometry and 24-h esophageal pH-impedance. Presence of pathological gastroesophageal reflux by esophageal pH-impedance studies, positive symptom index, symptom association probability, and symptom sensitivity indexes were necessary to proceed to the dietary intervention phase.

Dietary intervention

Patients were provided with psyllium (Mucofalk®, Dr. Falk PharmaGmbH, Germany) in sachets by 5.0 g and were instructed to use it three times a day (15 g per day that is an equivalent of 12.5 g of soluble dietary fiber). Psyllium was used in accordance with the manufacturer's recommendations: the content of the sachet was mixed with at least 150 mL of water, and the resulting suspension was taken as soon as possible, followed by an additional drink of liquid (1 cup). Besides psyllium supplementation, patients were advised to follow their usual diet. Formal interview on compliance with the study drug was performed at the end of the study and the number of used and unused sachets brought by the patient was counted.

No PPIs, H_2-hystamine receptors blockers, or prokinetics were allowed during the study. Antacid use was allowed when needed. It was recommended to use hydrotalcit 0.5 g (Rutacid, KRKA, Slovenia) no more than four times a day after meal. Patients were instructed to chew the tablet and then swallow it. Patients were asked to note the presence of heartburn, acid regurgitation and

Table 1 Study population

Population characteristics	Result
Total subjects in the study, *n*	30
Male/Female, *n*	18/12
Ethnic characteristic	Non-Hispanic Caucasians 100%
Age, yr, mean ± SD	34.7 ± 9.3
BMI, kg/m^2, mean ± SD	26.7 ± 6.9
Weight, kg, mean ± SD	82.5 ± 17.9
Waist/hip ratio, mean ± SD	0.91 ± 0.08
Smoking, yes, *n* (%)	6 (20)
Alcohol use, yes, *n* (%)	14 (46.7)
Alcohol, g/d, mean ± SD	1.1 ± 1.7
Dietary fiber intake, g/d, mean ± SD	6.0 ± 2.3
Hiatal hernia	
Presence, *n* (%)	16 (53.3)
Size, cm, mean ± SD	0.9 ± 0.5
Esophageal motility disorders per Chicago 3.0	
Ineffective esophageal motility, *n* (%)	14 (46.7)
Fragmented peristalsis, *n* (%)	9 (30.0)
Normal, *n* (%)	7 (23.3)
Mean stool frequency per week, mean ± SD	7.0 ± 2.0

BMI: Body mass index.

stool frequency during the treatment period.

Design of the study is shown on the Figure 1. Repeated 24-h esophageal pH-impedance, high resolution esophageal manometry, GERD-Q, and food frequency questionnaires were performed on the 10th day of treatment (end of treatment).

Main studied outcomes were GERD symptom presence during last 7 d, changes in the total GERD-Q score, number of reflux episodes (GER), their acidity and duration; lower esophageal sphincter (LES) mean resting pressure, minimal LES resting pressure, residual LES pressure, and percent of relaxation.

Statistical analysis

The obtained data were analyzed using standard software (Statistica 10, StatSoft Inc., United States). Wilcoxon matched pairs test of non-parametric module was used to assess changes of the studied parameters after the course of fiber supplementation in comparison to baseline. A *P* value of 0.05 was considered statistically significant.

Sample size calculation and power analysis. No similar studies were found in the literature to acquire data on the effect of psyllium on GERD symptoms and esophageal HRM and pH-impedance. We hypothesized that the main effect of the intervention would be a decrease in heartburn frequency. Our previous studies showed that GERD-Q score in the NERD patients group was (mean ± SD) 10.0 ± 1.5. To calculate sample size, we assumed that psyllium supplementation decrease GERD-Q score to 'normal' values (*i.e.*, less than 8) and choose a value of 7. Sample size calculation was performed using 1-Way ANOVA[34]. Effect size calculation was performed for every comparison. Value of size effect less than 0.2 indicated a small effect, 0.5 indicated a medium-sized effect, and 0.8 or higher indicated a large-size effect.

RESULTS

Thirty-six patients were enrolled in the study, and complete data from 30 were included in the final analysis (Table 1). One patient withdrew informed consent before day 0, and another patient was excluded due to non-compliance. One of the enrolled patients could not tolerate esophageal manometry, and in one case, there was no possibility to place the catheter due to narrow nasal passages and a deviated nasal septum. Migration of the pH-impedance probe was found in two patients during repeated examination. The data of mentioned these six patients were excluded from the final analysis (Figure 2).

Complete resolution of heartburn (*i.e.*, absence of the symptom during 7 consecutive days) was found in 18 of the 30 participants (60%) at the end-point (P = 0.0004) (Table 2). GERD-Q score decrease from (mean ± SD) 10.9 ± 1.7 at the baseline to 6.0 ± 2.3 at the end of treatment period (P < 0.001) (Table 2).

Mean lower esophageal sphincter resting pressure increased, but it did not reach statistical significance (mean ± SD: 22.6 ± 9.4 mm Hg *vs* 25.6 ± 11.8 mm Hg; P = 0.47). In the majority of patients, minimal resting pressure at rest as well as during functional tests with 10 water swallows was significantly decreased by the end of the study compared to the baseline (Table 2). No influence of the treatment on residual pressure and proportion of relaxation were found during the study.

The number of all but non-acid GERs significantly decreased (Table 2), resulting in a significant shortening of maximal reflux time (mean ± SD, 10.6 ± 12.0 at baseline to 5.3 ± 3.7 minutes at the end of treatment, P = 0.017). However, no significant changes in the mean esophageal pH and proportion of time with pH < 4 in the lower esophagus were found during the study.

Dietary fiber supplementation was well tolerated. No serious adverse event was registered during the study. Because of the primary indication of psyllium (laxative), significant increase in bowel movements was expected, but it was not necessary to withdraw treatment due to severe diarrhea (stool frequency per week, mean ± SD 7 ± 2 at baseline *vs* 8 ± 3 at the end of the treatment period, P = 0.00002).

Antacid use was registered in two out of 30 patients, and the number of taken tablets did not exceed the allowed maximum per day.

DISCUSSION

In this open-label prospective study, we demonstrated for the first time that intake of dietary fibers increases LES minimal resting pressure and decreases the number of acid, weakly-acid, and total refluxes. It was associated also with twice as low frequency of heartburn and GERD-Q score in patients with NERD. The effect of dietary

Table 2 Results of the study

	Baseline	EOT	*P* value
Symptoms' characteristics			
Presence of heartburn during 7 d, % of patients	93.3	40	0.000438
GERD-Q score, mean ± SD	10.9 ± 1.7	6.0 ± 2.3	0.000003
High resolution esophageal manometry (lower esophageal sphincter function)			
At rest, mean ± SD			
Mean resting pressure, mmHg	22.0 ± 9.4	26.5 ± 11.3	0.37
Minimal resting pressure, mmHg	5.41 ± 10.1	11.3 ± 9.4	0.023
Average, after 10 swallows of water, mean ± SD			
Mean resting pressure, mmHg	20.5 ± 9.5	22.0 ± 10.3	0.11
Minimal resting pressure, mmHg	14.1 ± 8.0	14.9 ± 6.4	0.008
Residual pressure, mmHg	7.5 ± 6.1	7.0 ± 5.4	0.94
% Relaxation	49.7 ± 15.0	51.3 ± 19	0.3
Esophageal 24-hrs pH-impedance, mean ± SD			
Number of refluxes	67.9 ± 17.7	42.4 ± 13.5	0.000002
Number of acid refluxes	43.2 ± 14.7	30.3 ± 15.3	0.002415
Number of weak acid refluxes	23.9 ± 11.7	11.3 ± 8.27	0.000016
Number of non-acid refluxes	0.7 ± 1.1	0.6 ± 1.7	0.34
Mean pH	5.9 ± 0.8	5.7 ± 0.9	0.06
% time pH < 4	5.6 ± 4.8	5.5 ± 7.57	0.20
Maximal reflux time, min	10.6 ± 12	5.3 ± 3.7	0.017
Number of high gastroesophageal refluxes (17 cm above LES), mean ± SD	23.1 ± 9.2	12.2 ± 6.6	0.000004
Gastric acid exposure			
Mean pH, mean ± SD	1.2 ± 0.29	1.3 ± 0.36	0.35

EOT: End of treatment period; LES: Lower esophageal sphincter.

interventions on the symptoms of gastroesophageal reflux disease is poorly studied. Available data are based predominantly on epidemiological studies. In the HUNT study in a Swedish population there was a negative correlation between coffee intake and reflux symptoms, with an approximate 40% decrease in risk among people who drank more than seven cups of coffee per day compared to those who drank less than one cup (OR = 0.6; 95%CI: 0.4-0.7)[35]. Also, a moderate and dose-dependent association between increasing frequency of meals of salted fish or meat and reflux symptoms was observed (*P* value for linear trend = 0.0007). The risk of reflux among people who ate salted food three times per week or more was higher by 50% compared with those who never ate salted food (OR = 1.5; 95%CI: 1.2-1.8). With increasing dietary fiber content in the predominantly consumed bread type (HUNT 2; cross sectional data), the risk of reflux significantly decreased (*P* value for linear trend, 0.0001). People who preferred to eat bread with 7% dry weight of dietary fibers or more had an approximately halved risk of having reflux symptoms compared with those who predominantly ate white, low fiber (1%-2%) content bread (OR = 0.5; 95%CI: 0.4-0.7)[35].

In a cross-sectional study by El-Serag *et al*[36], a non-significant trend for higher total caloric (energy) intake and lower fiber intake among persons with GERD symptoms was found. There was a dose-response relationship between GERD symptoms and total energy (calories per day) (*P* = 0.06), saturated fat (*P* = 0.04), cholesterol (*P* = 0.03), and fat servings (*P* = 0.06) intake. Specifically, saturated fat intake was positively associated with an increased risk of GERD symptoms. The authors noted that dietary fiber intake remained inversely associated with the risk of GERD symptoms in fully adjusted models, while associations between the other nutrients and GERD symptoms were not altered in direction or magnitude of the effect after adjusting for BMI, energy consumption, or demographics[36]. Surprisingly, despite solid epidemiological evidence of the possible protective effects of dietary fibers on GERD symptoms and risks of esophageal adenocarcinoma development[24,37,38], interventional studies supporting the effect of diet modification on esophageal function are still scarce, and we did not find any in which dietary fiber was used.

Assessment of nutritional factors affecting the presence of GERD symptoms showed that low dietary fiber intake is one of the typical features of GERD patients' diet[39,40]. Inverse medium strength correlation was found between dietary fiber intake and the presence of GERD (Spearman rank R = -0.26, *P* < 0.05)[40].

The significant influence of dietary fiber on esophageal motility and especially LES function in NERD patients was found in our study (Table 2). The function of LES after different meals was also studied by Sun *et al*[41] in eight GERD patients during the 2 h after a standard and fatty test meal. Increase in TLESRs was found after any test meal, but a decrease in resting pressure of LES was found only after the fatty meal, which was also associated with increased numbers of reflux episodes and percent of time with pH < 4. It was concluded that the combination of a decrease in LES pressure and TLESR is a major event that resulted in more severe and prolonged refluxes in GERD patients. These data correspond with the results of our study. Significant increase in minimal

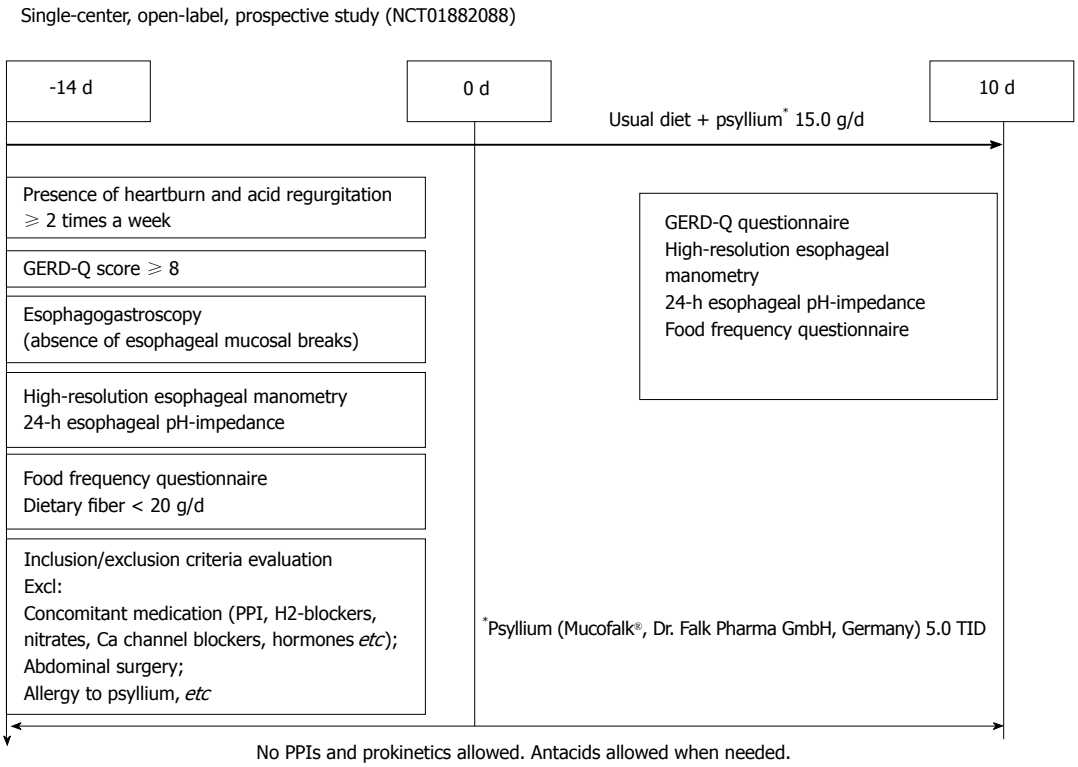

Figure 1 Design of the study. PPI: Proton pump inhibitor.

resting pressure of LES was found in our patients after treatment with dietary fibers, but there were no changes in percent of LES relaxation. Therefore, at least one component of anti-reflux barrier (LES pressure) was partly restored and, accordingly, the number of refluxes of all types has to be decreased, which was shown in our study (Table 2).

In this study, increased intake of dietary fiber significantly impacted the total number of refluxes and especially acid refluxes, according to the results of 24-h esophageal pH-impedance. The effect of different diets on esophageal acid exposure was assessed in a few studies. In one cross-over study, it was shown that esophageal acid exposure was greater during the high-calorie than low-calorie diet (mean, 8.6% ± 2.0% *vs* 5.2% ± 1.4% time pH < 4/24 h; $P < .01$). No difference was observed between the high-fat and low-fat diets [mean, 8.6% ± 2.0% *vs* 8.2% ± 1.6% time pH < 4/24 h; P = non-significant (NS)]. In contrast, the frequency of reflux symptoms was not affected by calorie density (median, 6; range, 2-12 *vs* median, 8; range, 2-13; P = NS) but was increased by high-fat content (median, 11; range, 5-18 *vs* median, 6; range, 2-12; $P < 0.05$)[42]. The effect of carbohydrate quote reduction (to < 20 g of carbohydrates a day) on esophageal acid exposure and symptoms of GERD within 1 week (3 to 6 d) was assessed in a prospective study. After the start of intervention, the percentage of time with pH < 4 decreased from 5.1% to 2.5% (P = 0.022), and Johnson-DeMeester score significantly reduced (mean ± SE of 34.7 ± 10.1 before the diet *vs* 14.0 ± 3.7 after initiating the diet; P = 0.023)[43]. The mean GERD Symptom Assessment Scale-Distress Subscale (GSAS-ds) score decreased from 1.28 to 0.72 (P = 0.0004), and, specifically, the severity score of the symptom "heartburn or burning pain inside the chest or breast bone" improved from 1.88 ± 0.23 prior to the diet to 0.88 ± 0.23 following initiation of the diet (P = 0.019). Unfortunately, only eight subjects of the same sex were enrolled in the study, and no data regarding the actual diet and amount of dietary fiber were provided by the authors.

The dose of the dietary fiber used in the study was chosen based on the on-label information, ethical considerations, safety reasons, and the need for dose standardization. The enrolled patients had very low basal dietary fiber intake (approximately 6.0 g/d, Table 1), therefore, supplementation with 12.5 g of soluble fiber a day during the study drew near the recommended daily allowance, according to national Russian guidelines (20 g/d)[44]. This dose of dietary fiber was far from that dose recommended in the United States (14 g/1000 kcal/d, using the energy guideline of 2000 kcal/d for women and 2600 kcal/d for men, the recommended daily dietary fiber intake is 28 g/d for women and 36 g/d for men)[45]. This difference may partly explain why the mean lower esophageal sphincter resting pressure increase did not reach statistical significance (Table 2). The effects of dietary fiber on GERD symptoms seen in epidemiological studies were dose-dependent, *i.e.* higher dose of consumed dietary fiber was associated with a lower risk of heartburn[35]. Efficacy and safety of higher dietary fiber doses in GERD patients need to be studied in a specially designed dose escalating trial.

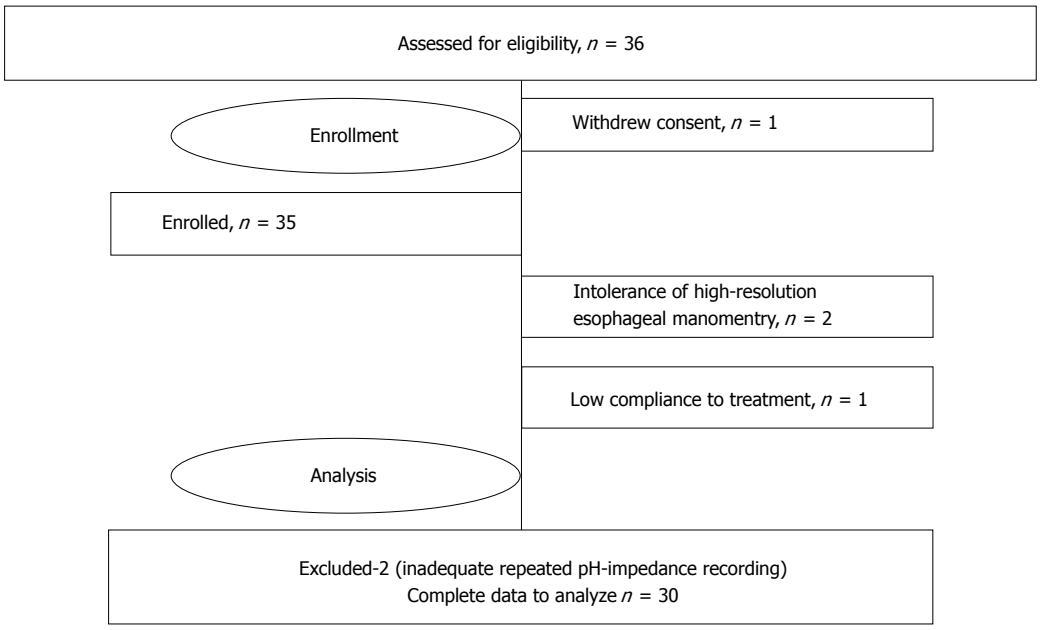

Figure 2 Patients screening and recruitment chart.

Since the recommended daily allowances differ around the world, it seems reasonable to confirm the obtained results in countries with different dietary habits.

Psyllium was chosen for the dietary intervention because the mentioned preparation is the only dietary fiber approved as a drug in Russia. The amount of dietary fiber in the drug is controlled, in contrast to food supplements were the quantity of psyllium may somewhat differ. According to the aim of the study, we needed to guarantee the amount of dietary fiber consumed to ensure the validity of the results.

The means of supplementation may also play an important role. A healthy diet is more readily accepted by patients than regular intake of drugs or food supplements[46,47]. However, in that case, the actual amount of the fiber consumed is more difficult to control.

The performed study has a number of limitations that were predictable at the phase of planning. For example, no previous data on the influence of psyllium on esophageal motility were available. Therefore, it was not possible to estimate the sample size necessary to achieve statistically significant results on the mean lower esophageal sphincter resting or residual pressure. We suppose that the results obtained here may help to plan further studies. Another limitation is the absence of a placebo-control. Due to the nature of psyllium and its preparation, it is almost impossible to produce a comparator of similar viscosity, solubility in water, and taste. Our study did, however, provide additional data for evidence-based modification of NERD-patient diet.

In conclusion, a fiber-enriched diet led to a significant increase of minimal lower esophageal sphincter resting pressure and a decrease of the number of gastroesophageal refluxes and frequency of heartburn per week in NERD patients with low dietary fiber intake. Psyllium 5.0 g TID was well tolerated by non-erosive GERD patients with low dietary fiber intake. Larger and placebo controlled studies are needed to confirm the obtained results.

ARTICLE HIGHLIGHTS

Background

Frequency of heartburn is negatively correlated with the amount of dietary fiber consumed according to epidemiological studies. Low dietary fiber intake is associated with decreased stomach and gut motility and delayed gastric emptying, which may contribute to the risk of gastroesophageal reflux. The ability of dietary fibers to bind nitric oxide contained in food may diminish its negative effect onto low esophageal sphincter pressure, but it has not been clinically proven yet. This is the first prospective trial demonstrating that an increase of dietary fiber consumed results in a significant increase of minimal esophageal resting pressure a decrease of the number of gastroesophageal refluxes and frequency of heartburn per week in patients with non-erosive gastroesophageal reflux disease (GERD) (NERD).

Research motivation

Reflux disease symptoms are associated with low consumption of dietary fiber, according to epidemiological studies. However, no studies were available to date that evaluated the effect of dietary fibers on esophageal motility and reflux pattern and there were no interventional studies demonstrating the effect of dietary fibers on GERD symptoms. For the first time, we showed that additional daily consumption of 12.5 g of soluble dietary fiber is associated with an increase in minimal lower esophageal sphincter resting pressure and a decrease in the number of gastroesophageal refluxes and frequency of heartburn per week in NERD.

Research objectives

The main objective of the study was to evaluate the effect of increased dietary fiber consumption on the number of gastroesophageal refluxes, esophageal acidity, the lower esophageal sphincter pressure, and clinical manifestations of NERD in patients with low dietary fiber intake.

Research methods

The study was conducted as a pilot single-center prospective trial with very strict inclusion criteria aimed to support the diagnosis and to exclude other reasons able to affect esophageal motility and NERD symptoms. Change

in GERD-Q questionnaire score, lower esophageal sphincter function by high resolution esophageal manometry, number of different types of gastroesophageal refluxes, and acid exposure time were assessed before and after patient diet modification (increased intake of dietary fiber). Data were analyzed using non-parametric statistics.

Research results

Our study is the first prospective trial demonstrating that increasing the amount of dietary fiber consumed results in an increase of minimal esophageal resting pressure and a decrease of the number of gastroesophageal refluxes and frequency of heartburn per week in patients with non-erosive GERD. Diet modification with additional psyllium (5.0 g TID) was well tolerated by non-erosive GERD patients with low dietary fiber intake.

Research conclusions

Our results are consistent with epidemiological studies that found an inverse correlation between the amount of dietary fibers consumed and symptoms of GERD. We demonstrated that diet modification with an addition of 12.5 of soluble fiber a day led to a decrease of GERD symptom frequency, an increase in lower esophageal sphincter resting pressure, and a decrease in the number of gastroesophageal refluxes. These findings are promising and suggest that nutritional interventions may be effective in GERD management.

Research perspectives

Well-planned trials are needed to examine further novel potential mechanisms of nutritional support for patients with esophageal disorders. Moreover, multicenter, placebo-controlled, dose-escalating trials are necessary to confirm our results, to establish the dose necessary to reach the optimal effect on esophageal motility and NERD symptoms, and to evaluate the effect of different types of dietary fibers.

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ORIGINAL ARTICLE

Prospective Study

Acute kidney injury in acute-on-chronic liver failure is different from in decompensated cirrhosis

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Abstract

AIM

To evaluate the differences in acute kidney injury (AKI) between acute-on-chronic liver failure (ACLF) and decompensated cirrhosis (DC) patients.

METHODS

During the period from December 2015 to July 2017, 280 patients with hepatitis B virus (HBV)-related ACLF (HBV-ACLF) and 132 patients with HBV-related DC (HBV-DC) who were admitted to our center were recruited consecutively into an observational study. Urine specimens were collected from all subjects and

the levels of five urinary tubular injury biomarkers were detected,including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), liver-type fatty acid binding protein (L-FABP), cystatin C (CysC), and kidney injury molecule-1 (KIM-1). Simultaneously, the patient demographics, occurrence and progression of AKI, and response to terlipressin therapy were recorded. All patients were followed up for 3 mo or until death after enrollment.

RESULTS

AKI occurred in 71 and 28 of HBV-ACLF and HBV-DC patients, respectively (25.4% *vs* 21.2%, P = 0.358). Among all patients, the levels of four urinary biomarkers (NGAL, CysC, L-FABP, IL-18) were significantly elevated in patients with HBV-ACLF and AKI (ACLF-AKI), compared with that in patients with HBV-DC and AKI (DC-AKI) or those without AKI. There was a higher proportion of patients with AKI progression in ACLF-AKI patients than in DC-AKI patients (49.3% *vs* 17.9%, P = 0.013). Forty-three patients with ACLF-AKI and 19 patients with DC-AKI were treated with terlipressin. The response rate of ACLF-AKI patients was significantly lower than that of patients with DC-AKI (32.6% *vs* 57.9%, P = 0.018). Furthermore, patients with ACLF-AKI had the lowest 90 d survival rates among all groups (P < 0.001).

CONCLUSION

AKI in ACLF patients is more likely associated with structural kidney injury, and is more progressive, with a poorer response to terlipressin treatment and a worse prognosis than that in DC patients.

Key words: Decompensated cirrhosis; Acute-on-chronic liver failure; Acute kidney injury; Biomarker; Etiology; Treatment; Prognosis

Core tip: Acute kidney injury (AKI) is common in acute-on-chronic liver failure (ACLF) and decompensated cirrhosis (DC) patients. Though ACLF and DC have been identified as two different diseases, the difference in AKI between these two diseases is rarely studied, and whether AKI should be handled in the same way in both diseases is still uncertain. This study combined multiple tubular injury biomarkers and has shown that AKI in patients with ACLF is distinctly different from in DC patients. AKI in ACLF patients is more likely to be caused by structural damage, and tends to be more progressive, with poorer response to terlipressin treatment and a worse prognosis.

Jiang QQ, Han MF, Ma K, Chen G, Wan XY, Kilonzo SB, Wu WY, Wang YL, You J, Ning Q. Acute kidney injury in acute-on-chronic liver failure is different from in decompensated cirrhosis. *World J Gastroenterol* 2018; 24(21): 2300-2310 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i21/2300.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i21.2300

INTRODUCTION

Acute kidney injury (AKI), including hepatorenal syndrome (HRS), is a common complication of patients with acute-on-chronic liver failure (ACLF) or decompensated cirrhosis (DC) and is always associated with poor outcome[1-3]. Previous studies have clearly demonstrated that acute-on-chronic liver failure and decompensated cirrhosis are two different diseases[4,5]. In patients with decompensated cirrhosis, the liver and extrahepatic organ failure usually occurs gradually over several weeks to several months on the basis of cirrhosis, and patients often have severe circulatory dysfunction. For acute-on-chronic liver failure, the liver failure often happens suddenly within 4 wk, in patients with either previously diagnosed or undiagnosed chronic liver disease and is usually associated with a precipitating even, and the systemic inflammatory response play an important role in the pathogenesis of organ failure[4,5]. However, the differences in acute kidney injury between patients with these two diseases are rarely studied, and it is uncertain whether AKI should be treated in the same way in these two diseases. A clear clarification on the differences in AKI between ACLF and DC patients will promote timely and more appropriate management of the patients.

Clinically, AKI can be divided into structural and functional kidney injury, prerenal azotemia and HRS are the most common causes of functional kidney injury, and acute tubular necrosis is the most common cause of structural renal impairment[6-8]. Accurate distinguishing the etiologies of AKI is critical as their treatments differ markedly[6-8]. In recent years, studies on kidney tubular injury biomarkers for early detection of AKI have garnered broad interest, several studies demonstrated that some of these biomarkers in urine are significantly increased in patients with structural kidney injury and have the potential to distinguish structural from functional AKI, the combination of these biomarkers can improve the accuracy of diagnosis[7-10]. Terlipressin is a vasoconstrictor and is widely used in the treatment of HRS. Previous studies have shown that it can improve renal function in most patients with HRS.However, it is ineffective in patients with structural kidney injury[11,12].

Furthermore, due to the high incidence of hepatitis B virus (HBV) infection, patients with HBV-ACLF account for over 80% of all ACLF patients in China[1]. Therefore, in this prospective study, we assessed the levels of five extensively studied urinary biomarkers of tubular damage, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), liver-type fatty acid binding protein (L-FABP), and cystatin C (CysC), to explore the etiological differences of AKI between HBV-ACLF and HBV-DC patients. Simultaneously, differences in the natural course of AKI, patient's response to terlipressin treatment and patient outcomes were also evaluated, aimed to clarify the differences in AKI between ACLF and DC patients.

MATERIALS AND METHODS

Patients

Consecutive patients with HBV-ACLF or HBV-DC who were admitted to Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology between December 2015 and July 2017 were enrolled in this observational study. This study was approved by the Ethics Committee of Tongji Hospital (TJ-C20151108), and written informed consents were obtained from all participants or their legal representatives. Two hundred and eighty patients with HBV-ACLF and 132 patients with HBV-DC were recruited and were divided into four groups according to the presence of ACLF, DC, and AKI, as follows: (1) Patients with DC without AKI (DC-non-AKI) group; (2) patients with ACLF without AKI (ACLF-non-AKI) group; (3) patients with both DC and AKI (DC-AKI) group; and (4) patients with both ACLF and AKI (ACLF-AKI) group. Patients with HBV-ACLF were diagnosed according to the definition of the Asian-Pacific Association for the Study of the Liver (APASL) 2014[5], this includes patients with previous HBV infection who had developed jaundice (total bilirubin $\geqslant$ 5 mg/dL) and coagulopathy (prothrombin activity (PTA) < 40% or INR $\geqslant$ 1.5) within 4 wk, and complicated by ascites and/or encephalopathy. HBV-DC patients were those with HBV-related cirrhosis, which were confirmed by a combination of clinical, imaging (computed tomography, magnetic resonance imaging, or ultrasonography) and endoscopic findings, presenting with significant signs of decompensation, such as ascites, hepatic encephalopathy, variceal bleeding, spontaneous bacterial peritonitis (SBP), or hepatorenal syndrome, but have not yet reached the ACLF diagnostic criteria, or have a history of liver function decompensation[13].

AKI was diagnosed according to the International Club of Ascites (ICA)-AKI criteria[3], as follows: an increase in serum creatinine by more than 0.3 mg/dL ($\geqslant$ 26.5 μmol/L) within 48 h or to more than 1.5 times the baseline value. The most recent serum creatinine result within the previous three months, or the serum creatinine result upon hospital admission, was considered as the baseline serum creatinine. AKI was categorized into three stages according to the ICA-AKI staging standard[3]: Stage 1 (AKI-1), an increase in serum creatinine to more than 0.3 mg/dL (26.5 μmol/L) or by 1.5 to 2 fold from baseline value; stage 2 (AKI-2), an increase in serum creatinine by 2 to 3 fold from baseline value; stage 3 (AKI-3), an increase in serum creatinine to more than 3 fold from baseline or need renal replacement therapy. The recovery or progression of AKI was evaluated at discharge and the patients were classified as no-change (if there was no change of AKI stage), recovery (if the patient reached a lower stage from the first recorded or acquired a normal renal function), or progression (if there was AKI stage deterioration to a higher stage or if the patient needed dialysis).

Twenty-four patients with mild chronic hepatitis B (CHB) and 20 health controls (HC) during the same period were also included as control groups. Our exclusion criteria included those patients with chronic kidney disease, obstructive uropathy, urinary tract infection, hepatocellular carcinoma or other malignancies, cirrhosis or liver failure without HBV infection, acute liver failure, previous kidney or liver transplantation, pregnancy, age < 18 or > 80 years.

All study participants were hospitalized and received anti-HBV therapy along with standard supportive treatment according to their individual indications. Patients with stage 2 or 3 AKI who do not respond to the diuretic withdrawal and plasma volume expansion with albumin and without apparent structural kidney injury had received terlipressin treatment according to the International Club of Ascites (ICA)-AKI recommendations[3]. Among them, 10 patients with ACLF-AKI and 6 patients with DC-AKI were treated with octreotide at the same time due to gastrointestinal bleeding or acute pancreatitis. Patient's response to terlipressin was assessed at the end of treatment, as follows: (1) No response, no regression of AKI; (2) partial response, AKI regression to a lower stage with serum creatinine decreased to $\geqslant$ 0.3 mg/dL (26.5 μmol/L) above the baseline value; or (3) full response, serum creatinine decreased to a value within 0.3 mg/dL (26.5 μmol/L) of the baseline value.

Patient demographics, clinical and laboratory data,and the natural course of AKI were recorded after enrollment, all patients were followed up for at least 3 mo or until death.

Specimen collection and biomarker measurement

Ten milliliter of fresh urine samples were collected on the day of enrollment and/or after AKI was confirmed. The samples were immediately centrifuged at 3000 rpm for 15 min at -4 ℃ and the supernatants were subsequently stored at -80 ℃ for future biomarker and creatinine measurements. Five urine samples were could not be collected due to either the patients' inability to cooperate or the presence of anuria. Samples from 24 CHB patients and 20 healthy controls (HC) were also collected.

The biomarkers of kidney tubular damage were measured using corresponding enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions: NGAL (BioPorto, Gentofte, Denmark), L-FABP (Hycultbiotech, Uden, The Netherlands), IL-18 (Medical and Biological Laboratories, Nagoya, Japan), CysC (R&D Systems, Minneapolis, MN), KIM-1 (R&D Systems, Minneapolis, MN).The ELISA methods and detection ranges for these biomarkers were as previously described[14,15]. All intra-assay and inter-assay variabilities were less than 10%. Urine creatinine was measured by enzyme colorimetry using an automatic biochemical analyzer (cobas8000, Roche Diagnostics, Mannheim, Germany).The concentrations of all urinary biomarkers were normalized to urinary creatinine to adjust for variations of urine concentration.

Statistical analysis

In this study, categorical variables were expressed as

Table 1 Baseline characteristics of hepatitis B virus-related acute-on-chronic liver failure and hepatitis B virus-related decompensated cirrhosis patients categorized according to the presence of acute kidney injury

Characteristics	HBV-DC		HBV-ACLF		*P* value[a]	*P* value[b]
	DC-non-AKI (*n* = 104)	DC-AKI (*n* = 28)	ACLF-non-AKI (*n* = 209)	ACLF-AKI (*n* = 71)		
Age (yr)[1]	51.4 ± 1.0	58.1 ± 2.2	44.2 ± 0.8	49.3 ± 1.3	0.002	< 0.001
Male (%)[3]	89 (85.6)	17 (60.7)	189 (90.4)	65 (91.5)	0.002	< 0.001
Cirrhosis (%)[3]	104 (100)	28 (100)	87 (41.6)	34 (47.9)	< 0.001	< 0.001
Complications						
Ascites (%)[3]	73 (70.2)	27 (96.4)	127 (60.8)	58 (81.7)	0.105	< 0.001
HE (%)[3]	6 (5.8)	1 (3.6)	13 (6.2)	14 (19.7)	0.06	0.006
GI bleeding (%)[3]	8 (7.7)	4 (14.3)	2 (1)	3 (4.2)	0.097	0.001
SBP (%)[3]	13 (12.5)	17 (60.7)	28 (13.4)	41 (57.7)	0.787	< 0.001
Pulmonary infection (%)[3]	11 (10.6)	8 (28.6)	14 (6.7)	23 (32.4)	0.638	< 0.001
Diabetes (%)[3]	10 (9.6)	3 (10.7)	17 (8.1)	10 (14.1)	1	0.492
Hypertension (%)[3]	6 (5.7)	4 (14.3)	13 (6.2)	8 (11.3)	0.736	< 0.212
Clinical and laboratory data						
ALT (U/L)[2]	40.5 (22-82)	33.5 (21-59.5)	134 (70.5-302)	136 (60.5-253.5)	< 0.001	< 0.001
AST (U/L)[2]	56 (39.3-88.7)	61 (39.5-104)	119 (78.5-207)	146 (62-277.5)	< 0.001	< 0.001
ALP (U/L)[2]	103 (82.3-137.8)	97 (70.8-120)	132 (110-162)	129 (101.5-155)	0.001	< 0.001
Serum bilirubin (mg/dL)[2]	2.8 (1.3-5.3)	4.1 (1.7-8.0)	17.5 (11.2-25)	25.7 (18.4-34)	< 0.001	< 0.001
Serum albumin (g/L)[2]	31.3 (27.05-34.4)	28.6 (24.1-33.7)	31.8 (29.2-34.4)	31.5 (28.7-34.6)	0.023	< 0.057
Serum creatinine (mg/dL)[2]	0.78 (0.68-0.87)	0.97 (0.81-1.23)	0.68 (0.6-0.81)	0.94 (0.74-1.26)	0.665	< 0.001
BUN (mmol/L)[2]	4.0 (3.3-5.2)	12.8 (8.0-17.8)	3.5 (2.8-4.3)	11.2 (8.2-18)	0.905	< 0.001
eGFR (mL/min/1.73 m^2)[2]	104 (92.8-115.1)	45.9 (40-59.5)	113.9 (102.8-124.7)	42.7 (27.4-58.5)	0.164	< 0.001
Serum sodium (mmol/L)[2]	138.5 (134.7-141)	135.4 (133.2-138.4)	137.3 (134.7-139.4)	130 (126.4-133.9)	0.001	< 0.001
Serum potassium (mmol/L)[2]	4.0 (3.6-4.3)	3.9 (3.4-4.3)	4.1 (3.6-4.4)	3.6 (3.1-4.5)	0.487	< 0.001
INR[2]	1.45 (1.28-1.81)	1.65 (1.48-2.14)	1.89 (1.6-2.65)	2.81 (1.98-3.86)	< 0.001	< 0.001
Leukocyte count (× 10^9/L)[2]	3.6 (2.5-5.0)	4.1 (3.1-6.6)	5.9 (4.4-8.4)	10.0 (6.0-13.3)	< 0.001	< 0.001
PLT (× 10^9/L)[2]	61.3 (45.3-104.8)	67.5 (39.3-89.3)	95.2 (64.5-140.5)	79 (47-115.5)	0.058	< 0.001
Hemoglobin (g/L)[2]	114 (94.5-126)	95.5 (75.75-112)	123 (107.5-136)	115 (100.5-131.5)	< 0.001	< 0.001
MAP (mmHg)[1]	82.9 ± 1.1	75.9 ± 1.5	86.7 ± 0.7	76.7 ± 1.1	0.921	< 0.001
HBV-DNA ($\log_{10}$)[2]	4.5 (2.7-6.3)	4.1 (2.8-6.1)	5.4 (3.7-7.1)	5.3 (3.5-7.2)	0.043	0.013
Child-Pugh score[2]	9 (7-11)	11 (8-12)	11 (9-12)	12 (11-13)	0.061	< 0.001
MELD score[2]	13 (8.1-16)	19.7 (16.2-25.3)	21.2 (19-25)	34.5 (29.2-41.6)	< 0.001	< 0.001

[1]Means ± SD, compared by Student's *t* test or one-way ANOVA test; [2]Median (IQR), compared by Mann-Whitney *U* test or Kruskal-Wallis test; [3]Number (percentage), compared by fisher's exact test or chi-square test; [a]DC-AKI group *vs* ACLF-AKI group; [b]Compared among all groups. SD: Standard deviation; IQR: Inter-quartile range; DC: Decompensated chirrhosis; ACLF: Acute-on-chronic liver failure; AKI: Acute kidney injury; HE: Hepatic encephalopathy; GI: Gastrointestinal; SBP: Spontaneous bacterial peritonitis; ALT: Alanine amino transaminases; AST: Aspartate transaminases; ALP: Alkaline phosphate; BUN: Blood urea nitrogen; eGFR: Estimated glomerular filtration rate; INR: International normalized ratio; MAP: Mean arterial pressure; MELD: Model of end-stage liver disease score.

frequencies and percentages, and were compared using Fisher's exact test or the chi-square test. Continuous variables were reported as mean ± SD for normally distributed variables and were compared using the Student's t test or one-way ANOVA testing. Continuous variables with non-normal distributions were presented as medians with interquartile ranges (IQR) and were compared using the Mann-Whitney *U* test or the Kruskall-Wallis test. The cumulative survival rates at 90 d were estimated using the Kaplan-Meier method and were compared by the Log-rank test. A Cox proportional-hazards model, adjusted for potential confounders, was used to estimate the effects of DC, ACLF and AKI on 90-day mortality. All analyses in this study were conducted using IBM SPSS Statistics 23.0 and $P < 0.05$ (two-sided) was considered statistically significant.

RESULTS

Patient's characteristics and demographics

A total of 280 patients with HBV-ACLF and 132 with HBV-DC were enrolled. During admission or hospitalization, 71 and 28 patients developed AKI in HBV-ACLF and HBV-DC groups, respectively (25.4% *vs* 21.2%, $P = 0.358$). Baseline and hospitalization characteristics of patients with HBV-ACLF or HBV-DC are shown in Tables 1 and 2.

Patients in the ACLF-AKI group had the highest Model for End-stage Liver Disease (MELD) score, serum bilirubin levels, INR, and leukocyte counts and the lowest serum sodium levels. In contrast, patients with DC-AKI had the lowest serum albumin and hemoglobin levels. Prevalences of ascites, SBP, and pulmonary infection was noted to be higher among AKI patients compared to those without AKI, but there were no differences between the ACLF-AKI and DC-AKI groups. Hepatic encephalopathy (HE) was more common in ACLF-AKI patients than in DC-AKI patients.

The levels of tubular damage biomarkers

The concentrations of NGAL, CysC, L-FABP, IL-18 in urine were found to be significantly elevated in patients

Table 2 Clinical characteristics of hepatitis B virus-related acute-on-chronic liver failure and hepatitis B virus-related decompensated cirrhosis patients after enrollment

Characteristics	HBV-DC		HBV-ACLF		*P* value[a]	*P* value[b]
	DC-non-AKI (*n* = 104)	DC-AKI (*n* = 28)	ACLF-non-AKI (*n* = 209)	ACLF-AKI (*n* = 71)		
Hospitalization (d)[1]	13 (8-20)	12.5 (9-18.3)	26 (17-43)	16 (10.5-33)	0.144	< 0.001
Complications						
Ascites (%)[2]	80 (76.9)	28 (100)	141 (67.5)	67 (94.4)	0.570	< 0.001
HE (%)[2]	8 (7.7)	3 (10.7)	41 (19.6)	31 (43.7)	< 0.001	< 0.001
GI bleeding (%)[2]	11 (10.6)	5 (17.9)	5 (2.4)	6 (8.5)	0.151	0.002
SBP (%)[2]	22 (21.2)	19 (67.9)	68 (32.5)	47 (66.2)	0.872	< 0.001
Pulmonary infection (%)[2]	20 (19.2)	8 (28.6)	43 (20.6)	23 (32.4)	0.944	0.134
Serum creatinine (mg/dL)[1]						
Baseline	0.78 (0.68-0.87)	0.97 (0.81-1.23)	0.68 (0.6-0.81)	0.94 (0.74-1.26)	0.665	< 0.001
Peak	0.84 (0.74-0.96)	1.69 (1.44-2.07)	0.83 (0.7-0.94)	1.99 (1.63-2.57)	0.028	< 0.001
Final	0.76 (0.66-0.87)	1.05 (0.77-1.48)	0.74 (0.64-0.85)	1.48 (0.98-2.32)	0.014	< 0.001
Treated with terlipressin (%)[2]	-	19 (67.9)	-	43 (60.6)	0.499	-
Treatment time[1]	-	5 (3-9)	-	6 (3-9)	0.023	-
30-d mortality[2]	7 (6.7)	9 (32.1)	38 (18.2)	42 (59.2)	0.015	< 0.001
90-d mortality[2]	10 (9.6)	14 (50)	69 (33)	51 (71.8)	0.039	< 0.001

[1]Median (IQR), compared by Mann-Whitney *U* test or Kruskal–Wallis test; [2]Number (percentage), compared by fisher's exact test or chi-square test; [a]DC-AKI group *vs* ACLF-AKI group; [b]Compared among all groups. SD: Standard deviation; IQR: Inter-quartile range; HE: Hepatic encephalopathy; GI: Gastrointestinal; SBP: Spontaneous bacterial peritonitis.

with ACLF-AKI, which were markedly higher than those in the DC-AKI group and the groups without AKI,but there was no significant difference in the levels of these biomarkers between DC-AKI and non-AKI patients. The level of urinary KIM-1 was significantly higher in ACLF-AKI patients than in those without AKI, while no difference was observed between ACLF-AKI and DC-AKI groups (Figure 1).

Recovery and progression of AKI

At the time of AKI diagnosis, there were 33 (46.5%) AKI-1, 28 (39.4%) AKI-2, and 10 (14.1%) AKI-3 patients in the ACLF-AKI group and 17 (60.7%) AKI-1, 9 (32.1%) AKI-2, and 2 (7.2%) AKI-3 patients in the DC-AKI group (P = 0.396) (Figure2A). However, for the peak stages of AKI, these proportions were significantly different among ACLF-AKI and DC-AKI patients: there were 23 (32.4%) AKI-1, 22 (31%) AKI-2, and 26 (36.6%) AKI-3 patients in the ACLF-AKI group and 13 (46.4%) AKI-1, 12 (42.9%) AKI-2, and 3 (10.7%) AKI-3 patients in the DC-AKI group (P = 0.039) (Figure 2B). Next, we assessed the progression of AKI at discharge and found a higher proportion of patients with AKI progression in the ACLF-AKI group than in the DC-AKI group (49.3% *vs* 17.9%, P = 0.013) (Figure 2C).

Patients' response to terlipressin treatment

There were 43 and 19 patients treated with terlipressin in the ACLF-AKI and DC-AKI groups, respectively (60.6% *vs* 67.9%, P = 0.499). At the end of treatment, there were 27 (62.8%) non-responders, 2 (4.7%) partial responders, and 14 (32.6%) full responders in the ACLF-AKI group and 5 (26.3%) non-responders, 3 (15.8%) partial responders, and 11 (57.9%) full responders in the DC-AKI group. The response rate in the ACLF-AKI group was significantly lower than that in the DC-AKI group (P = 0.018) (Figure2D).

Next, we used logistic regression analysis to determine factors associated with the response to terlipressin treatment. A univariate analysis showed that DC patients with lower leukocyte count, serum creatinine, INR, total bilirubin (TBIL) and MELD scores, without the occurrence of HE had a good response to terlipressin. The levels of TBIL, INR, serum creatinine and MELD scores were closely related to the patient's grouping, therefore were excluded from multivariate analysis. Among the parameters for multivariate analysis including patient's grouping (DC or ACLF), HE, and leukocyte count, patient's grouping (DC or ACLF) was independently associated with treatment response. Patients with ACLF-AKI were the poorest responders of terlipressin treatment (Table 3).

Outcomes

Survival rates at 90 d were significantly decreased in patients with AKI in comparison with those without. Patients with ACLF-AKI had the lowest survival rates among all groups (P < 0.001) (Figure3). A total of 14 patients received liver transplantation. One of the fourteen patients had AKI before transplantation and this patient survived until a 90 d follow-up. Five patients (2 patients with DC and 3 patients with ACLF) were lost to follow-up. All patients with mild CHB survived at 90 d follow-up.

To further assess the effects of AKI, ACLF and DC on 90-day mortality, several factors (age, presence of ascites, HE, SBP, and leukocyte count) that were associated with mortality in the univariate analysis were adjusted in a Cox proportional hazards model (Table 4). ACLF-AKI patients had a highest death risk [HR 7.986 (3.823-16.683)], markedly higher than that in

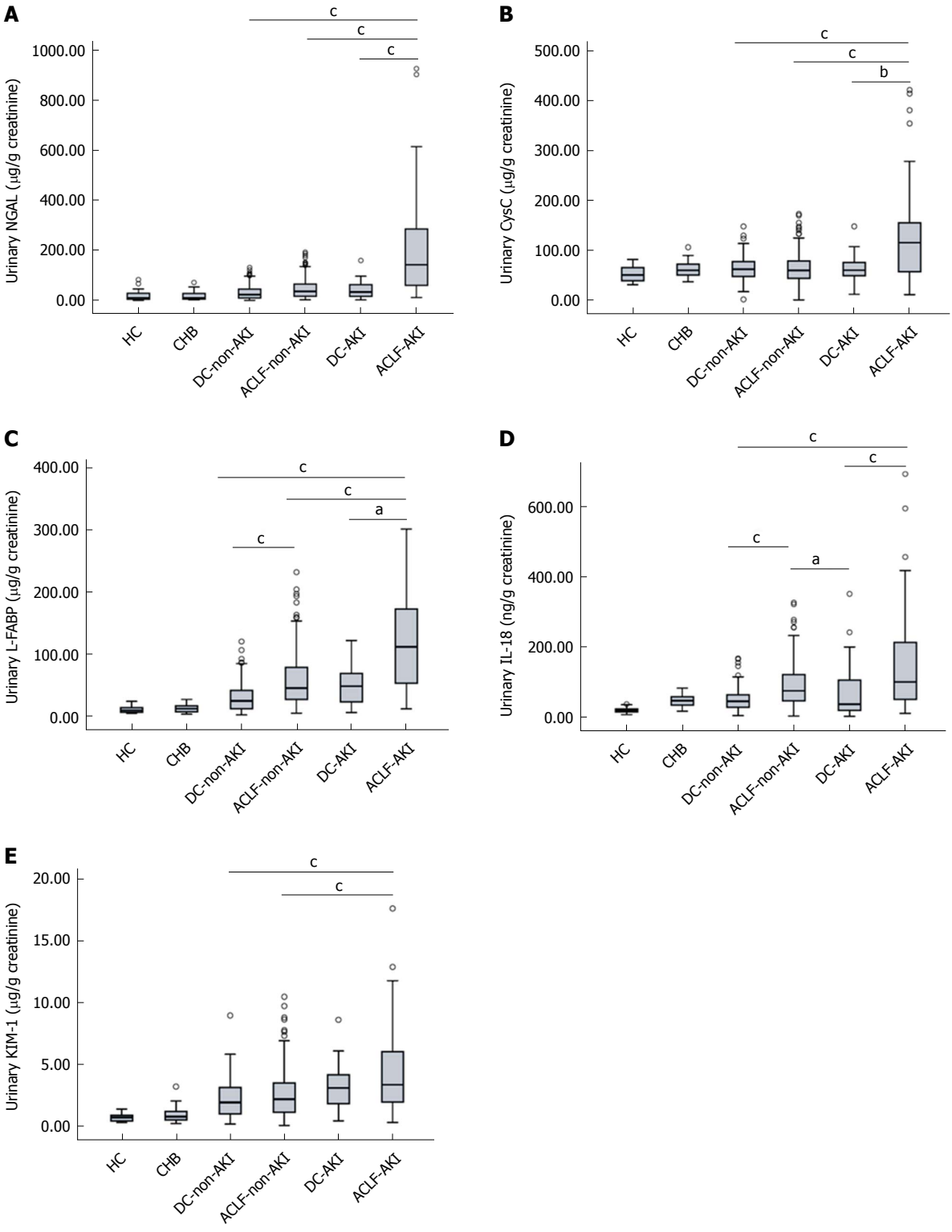


Figure 1 Box-plot of urinary tubular damage biomarkers levels in different groups. A: Urinary NGAL; B: Urinary CysC; C: Urinary L-FABP; D: Urinary IL-18; E: Urinary KIM-1. The boxes in each graph represents the median (middle line), 25th percentile (bottom line) and 75th percentile (top line) values, whereas lower and upper whiskers represent data within1.5 IQR of the lower quartile and upper quartile, respectively. Circles represent outliers. Kruskal-Wallis test were used for all comparison and $P < 0.05$ were considered as have statistical significance, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. ACLF: Acute-on-chronic liver failure; DC: Decompensated cirrhosis; AKI: Acute kidney injury; CHB: Chronic hepatitis B; HC: Healthy controls; NGAL: Neutrophil gelatinase-associated lipocalin; CysC: Cystatin C; L-FABP: Liver-type fatty acid binding protein; IL-18: Interleukin-18; KIM-1: Kidney injury molecule-1.

other groups. The risk of death was also higher in DC-AKI patients [HR 4.674 (1.977-10.943)] than those in ACLF-non-AKI and DC-non-AKI individuals. In addition, older age and the presence of HE and ascites were also

Table 3 Univariate and multivariate logistics regression analysis to assess factors associated with the response to terlipressin treatment

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age	1.024 (0.975-1.075)	0.344		
Gender	0.35 (0.090-1.357)	0.129		
Grouping (DC/ACLF)	0.282 (0.087-0.913)	0.035	0.282 (0.087-0.913)	0.035
Baseline serum creatinine	1.074 (0.417-2.77)	0.882		
Peak serum creatinine	0.499 (0.268-0.930)	0.029		
Cirrhosis	1.50 (0.513-4.385)	0.459		
HE	0.318 (0.103-0.981)	0.046	-	0.148
GI bleeding	1.091 (0.262-4.537)	0.905		
Ascites	0.735 (0.044-12.330)	0.831		
SBP	0.452 (0.125-1.633)	0.226		
Pulmonary infection	0.970 (0.324-2.904)	0.956		
ALT	0.997 (0.993-1.001)	0.153		
AST	0.997 (0.993-1.002)	0.095		
Serum albumin	0.986 (0.895-1.1087)	0.782		
Serumbilirubin	0.956 (0.917-0.996)	0.032		
Serum sodium	1.071 (0.986-1.163)	0.103		
INR	0.462 (0.260-0.823)	0.009		
Leukocyte count	0.903 (0.816-0.999)	0.048	-	0.180
MAP	0.998 (0.937-1.062)	0.944		
Child-Pugh score	0.809 (0.608-1.076)	0.146		
MELD	0.921 (0.870-0.975)	0.004		
Treatment time	1.020 (0.978-1.065)	0.352		

DC: Decompensated chirrhosis; ACLF: Acute-on-chronic liver failure; HE: Hepatic encephalopathy; GI: Gastrointestinal; SBP: Spontaneous bacterial peritonitis; ALT: Alanine amino transaminases; AST: Aspartate transaminases; INR: International normalized ratio; MAP: Mean arterial pressure; MELD: Model of end-stage liver disease score.

Table 4 Cox proportional-hazards model to assess the 90 d death risk

Variables	90-d mortality	
	HR (95%CI)	*P* value
Age	1.022 (1.005-1.039)	0.010
Ascite	2.120 (1.075-4.178)	0.030
HE	5.342 (3.654-7.808)	< 0.001
DC without AKI	Reference	-
ACLF without AKI	3.449 (1.684-7.064)[1]	0.001
DC with AKI	4.674 (1.977-10.943)[1]	< 0.001
ACLF with AKI	7.986 (3.823-16.683)[1]	< 0.001

[1]The death risk of patients with DC without AKI were set as reference, HR were adjusted by age, presence of ascites, HE, SBP and leukocyte count. HR: Hazards ratio; HE: Hepatic encephalopathy; SBP: Spontaneous bacterial peritonitis; DC: Decompensated chirrhosis; ACLF: Acute-on-chronic liver failure; AKI: Acute kidney injury.

associated with 90 d mortality.

DISCUSSION

This study was conducted to explore the etiology, natural course and prognostic differences of AKI between patients with HBV-ACLF and HBV-DC. The response to terlipressin was also assessed between the two groups. We have demonstrated that the structural tubular damage is the dominant pathophysiological mechanism of AKI during the course of ACLF-AKI. We have also showed that AKI in HBV-ACLF patients were more progressive and have a lower response rate to terlipressin treatment as well as a worse prognosis compared with that in HBV-DC patients.

To the best of our knowledge, there is only one published study by Maiwall *et al*[16] that reported differences in AKI between ACLF and DC patients. In that study, patients with ACLF-AKI were found to be more likely to have structural kidney injury, which had a greater possibility to resolve despite of the faster progression and poorer prognosis compared to patients with DC. However, the majority of patients in that study were caused by alcoholic cirrhosis and AKI were classified based on microscopic urinalysis[16], which cannot accurately distinguish the type of renal injury in some cases[17,18]. Current study is the first one to investigate differences in AKI between HBV-ACLF and HBV-DC patients by evaluating of the levels of novel tubular damage biomarkers and comparing the patients' response to terlipressin treatment in different groups.

Accumulating evidences has shown that biomarkers of renal tubular injury in urine can distinguish between structural and functional renal impairment, though the specific biomarkers for differential diagnosis and their effect size remain controversial[7,8]. Fagundes *et al*[10] have previously shown that NGAL levels in urine could distinguish structural and functional kidney injury effectively. Ariza *et al*[19] also found that urinary NGAL is a good biomarker for differential diagnosis, followed by IL-18, but CysC and KIM-1 were found less useful for this purpose. Belcher *et al*[7] studied five biomarkers (NGAL, IL-18, L-FABP, KIM-1 and albumin) in their research and concluded that a combination of all those biomarkers significantly improved accuracy in the differentiation of

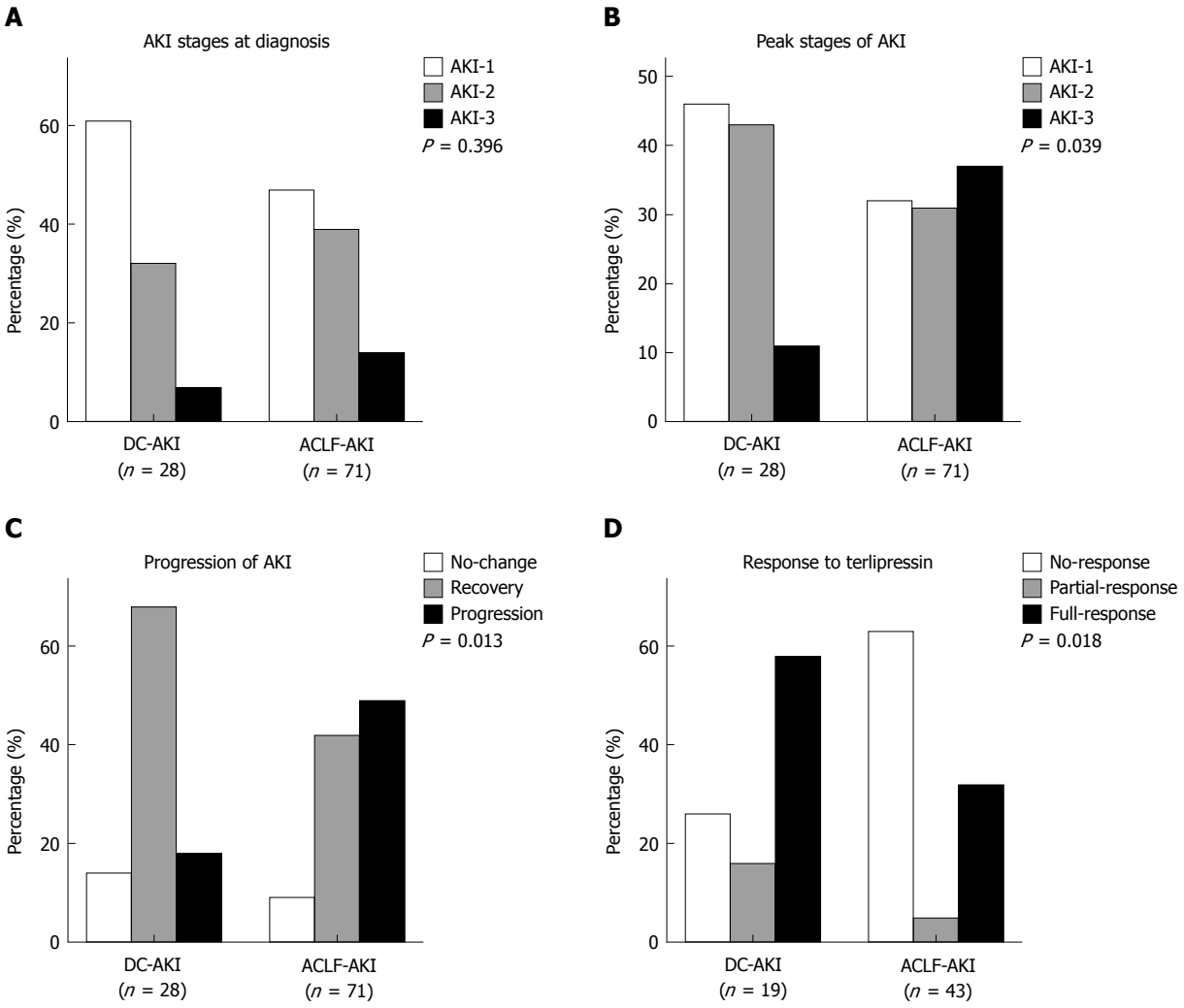
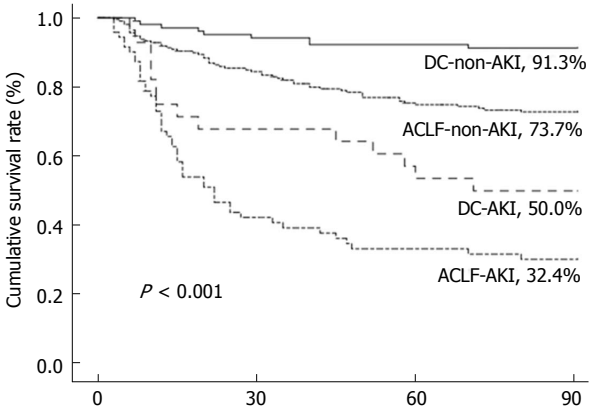


Figure 2 Acute kidney injury staging, progression and the response to terlipressin in ACLF-AKI and DC-AKI patients. A: AKI stages at diagnosis ($P = 0.396$); B: Peak stages of AKI ($P = 0.039$); C: Progression of AKI ($P = 0.013$); D: Patient's response to terlipressin ($P = 0.018$). All analyses compared by fisher's exact test or chi-square test, $P < 0.05$ were considered as have statistically significant.

structural and functional kidney injury compared with any single biomarker alone.

In the current study, five of the most extensively studied biomarkers (NGAL, CysC, L-FABP, IL-18, and KIM-1) were evaluated. Four (NGAL, CysC, L-FABP, and IL-18) of these biomarkers levels in urine were markedly elevated in ACLF-AKI patients, but not in DC-AKI patients and those without AKI. According to the findings of previous studies, the results of current study drove us to the hypothesis that AKI in HBV-ACLF patients is more likely to be caused by structural kidney injury than in HBV-DC patients, and our findings are consistent with that of Maiwall *et al*[16]. In addition to Maiwall's findings, we have further revealed that AKI is not only more progressive in HBV-ACLF patients but also associated with poor recovery.

In patients with DC, organ hypoperfusion due to progressive hemodynamic dysfunction caused by serious splanchnic vasodilation is considered a major cause of AKI. Patients with AKI usually have a lower mean arterial pressure (MAP)[2,20]. Similarly, we found that MAP was significantly lower in the DC-AKI group than in patients without AKI. There was no significant difference in MAP levels between the ACLF-AKI and DC-AKI groups, which was expected because of the similar but severe hemodynamic changes in ACLF and DC[20,21]. Previous studies have reported that the systemic inflammatory response plays a more important role than hemodynamic dysfunction in the pathogenesis of ACLF and organ failure, and these patients usually have elevated levels of pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs)[20,21]. These inflammatory mediators can directly or indirectly lead to microcirculation dysfunction, oxidative stress, mitochondrial energy metabolism disorders, and eventually renal tubular cell apoptosis and necrosis[22,23]. IL-18 is not only a biomarker of kidney injury but also an inflammatory mediator, and the levels of IL-18 in urine were significantly higher in patients with ACLF-AKI in this study. We also found significantly higher leukocyte counts in patients with ACLF, especially in those with ACLF-AKI. The different pathogeneses of ACLF and DC



Number at risk		Follow-up time (d)		
DC-non-AKI	104	97	95	94
ACLF-non-AKI	209	171	147	140
DC-AKI	28	19	16	14
ACLF-AKI	71	29	22	20

Figure 3 Kaplan-Meier curves shows the cumulative survival rates of acute-on-chronic liver failure and decompensated cirrhosis patients categorized accorrding to the presence of acute kidney injury. Survival estimates were compared by log-rank test, $P < 0.05$ was considered statistically significant. ACLF: Acute-on-chronic liver failure; DC: Decompensated cirrhosis; AKI: Acute kidney injury.

may explained the hypothesis that there is a difference in the etiology and natural course of AKI between these two disease states. In addition, previous studies have found that hyperbilirubinemia is one of the causes of structural renal injury in patients with liver disease[24,25]. The level of serum bilirubin in patients with ACLF was significantly higher than that in DC patients, this may also contribute to the differences in AKI between these two diseases.

Terlipressin is a vasoactive agent and has been widely used for the treatment of HRS[11,26]. Several previous studies have demonstrated that the use of terlipressin significantly improves renal function and survival in patients with decompensated cirrhosis[11,26]. However, research on the use of terlipressin to treat AKI in ACLF patients is limited. Jindal *et al*[27] reported that only 35% of patients with ACLF-AKI responded to terlipressin, which is lower than 40%-60% responders in DC-AKI as reported by other investigators. In this study, we also found that the response rate of the ACLF-AKI group was significantly lower than that of the DC-AKI group, and having HBV-related ACLF was an independent predictor of poor response to terlipressin. As terlipressin is ineffective in patients with structural renal impairment, and our study found that the levels of biomarkers that represent structural renal impairment in patients with ACLF-AKI was significantly higher than that in patients with DC-AKI, we considered the low response rate of terlipressin treatment in ACLF-AKI patients is associated with a higher proportion of structural kidney damage in these patients. In addition, previous studies have shown that high serum bilirubin levels are associated with a low response to terlipressin treatment, and elevated serum bilirubin levels are associated with the development of structural kidney injury[24,25,28,29]. Serum bilirubin levels were significantly higher in patients with ACLF-AKI than in DC-AKI patients in this study, further explaining our results. Although some of patients recieved octreotide, there was no significant difference in the proportion of patients receiving octreotide between the two groups.

There is persuasive evidence that AKI is associated with high mortality in patients with liver disease[30,31]. Similarly, we also found that survival rates were significantly lower in patients with AKI than those without. Moreover, it is interesting that survival rates in the ACLF-AKI group were significantly lower than those in the DC-AKI group. Many studies had demonstrated that the mortality of patients with AKI is stage-dependent and closely related to the etiologies of AKI[1,32,33]. Singer *et al*[34] reported that patients with structural kidney injury were usually associated with poor prognosis. Nadim *et al*[35] also showed that the presence of structural kidney injury was associated with higher mortality. A higher proportion of stage 2 or 3 AKI in HBV-ACLF patients was observed in this current study and which is more likely to be caused by structural kidney injury. This may explain the lower survival rates in ACLF-AKI pateints.

Although this is a prospective observational study with a large series of patients, there are still limitations. First, our findings cannot be further verified,as it is impractical to obtain kidney biopsies from most of the AKI patients in this serious condition. In addition, all patients in our study were enrolled from a single-center in China,there may be a certain selection bias. A multi-center prospective study needed for further investigation. Finally, this sutdy mainly focuses on HBV-related ACLF and DC patients. One should consider the definitions and etiology differences when interpret these results into western patients , where alcoholism constitutes the major etiology of ACLF (type A non-cirrhosis, type B with compensated cirrhosis, type C with decompensated cirrhosis) and DC[4].

In conclusion, this study demonstrated that AKI in patients with HBV-ACLF is distinctly different from that in HBV-DC patients. In patients with HBV-ACLF, AKI was more likely to be due to structural kidney injury, tended to be more progressive, with a lower response rate to terlipressin therapy and a poorer prognosis compared with those in DC-AKI patients. Accurate differentiating the causes of AKI is critical, and AKI in patients with HBV-ACLF or HBV-DC should be managed in different ways. Further studies are required to validate these findings.

ARTICLE HIGHLIGHTS

Research background

Acute kidney injury (AKI) is a common and serious complication of acute-on-chronic liver failure (ACLF) and decompensated cirrhosis (DC). Previous studies have been clearly established that the acute-on-chronic liver failure and decompensated liver cirrhosis are two different diseases.However, the differences in acute kidney injury among patients with these two diseases are

rarely studied and whether AKI should be managed in the same way in patients with these two diseases is still uncertain.

Research motivation

Clinically, the treatment of patients with different types of renal impairment is significantly different. A clear clarification on the differences in AKI between ACLF and DC patients will promote timely and more appropriate management of the patients.

Research objectives

This study was conducted to clarify the differences in AKI between hepatitis B virus (HBV)-ACLF and HBV-DC patients, including the differences in the etiology of AKI, natural course, patient's response to terlipressin and prognosis.

Research methods

This study is a prospective observational study, patients with HBV-ACLF and HBV-DC who were admitted to our hospital between 2015.12 and 2017.7 were consecutively recruited. Urine specimens of all patients were collected at the time of admission and when AKI was diagnosed, and the levels of five tubular injury biomarkers in urine were detected. Simultaneously, the demographic data, natural course of AKI, patient's response to terlipressin treatment and patient outcomes were recorded.

Research results

Patients with ACLF-AKI have significantly higher urinary biomarker levels than those with DC-AKI or without AKI. There was a higher proportion of patients with AKI progression in ACLF-AKI patients than in DC-AKI patients (49.3% *vs* 17.9%, $P = 0.013$). Forty-three patients with ACLF-AKI and 19 patients with DC-AKI were treated with terlipressin, the response rate to terlipressin was significantly lower in patients with ACLF-AKI than in patients with DC-AKI (32.6% *vs* 57.9%, $P = 0.018$). In addition, patients in the ACLF-AKI group had the lowest survival rate at 90 d among all groups ($P < 0.001$).

Research conclusions

Our study demonstrated that AKI in patients with HBV-ACLF is distinct different from in HBV-DC patients.In HBV-ACLF patients, AKI is more likely to be caused by structural damages and tends to be more progressive, with a poorer response to terlipressin and a worse prognosis than in HBV-DC patients.

Research perspectives

Our results suggest that AKI occurring in patients with HBV-ACLF or HBV-DC should be managed in different ways. Large-scale multi-center studies are required to validate these findings, and the differences in AKI between patients with ACLF and DC caused by other etiologies still need to be further studied.

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META-ANALYSIS

Intralesional steroid is beneficial in benign refractory esophageal strictures: A meta-analysis

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Abstract

AIM

To analyze the effect of intralesional steroid injections in addition to endoscopic dilation of benign refractory

esophageal strictures.

METHODS

A comprehensive search was performed in three databases from inception to 10 April 2017 to identify trials, comparing the efficacy of endoscopic dilation to dilation combined with intralesional steroid injections. Following the data extraction, meta-analytical calculations were performed on measures of outcome by the random-effects method of DerSimonian and Laird. Heterogeneity of the studies was tested by Cochrane's Q and I^2 statistics. Risk of quality and bias was assessed by the Newcastle Ottawa Scale and JADAD assessment tools.

RESULTS

Eleven articles were identified suitable for analyses, involving 343 patients, 235 cases and 229 controls in total. Four studies used crossover design with 121 subjects enrolled. The periodic dilation index (PDI) was comparable in 4 studies, where the pooled result showed a significant improvement of PDI in the steroid group (MD: -1.12 dilation/month, 95%CI: -1.99 to -0.25 P = 0.012; I^2 = 74.4%). The total number of repeat dilations (TNRD) was comparable in 5 studies and showed a non-significant decrease (MD: -1.17, 95%CI: -0.24-0.05, P = 0.057; I^2 = 0), while the dysphagia score (DS) was comparable in 5 studies and did not improve (SMD: 0.35, 95%CI: -0.38, 1.08, P = 0.351; I^2 = 83.98%) after intralesional steroid injection.

CONCLUSION

Intralesional steroid injection increases the time between endoscopic dilations of benign refractory esophageal strictures. However, its potential role needs further research.

Key words: Intralesional steroid; Meta-analysis; Benign refractory esophageal stricture; Dilation

Core tip: Benign refractory stricture can be a very challenging pathology, which requires regular endoscopic dilations. Results of this meta-analysis suggest that endoscopic intralesional steroid injection significantly decreases the frequency of the endoscopic dilations in benign refractory esophageal strictures. In addition, there are very few and mild complications reported in association with this method. We believe that the benefits of intralesional steroid in the treatment of benign refractory stricture overweigh its risks. However, further research would be essential on this treatment method, as there are no data concerning its efficacy and safety in different etiologies of refractory esophageal strictures.

Szapáry L, Tinusz B, Farkas N, Márta K, Szakó L, Meczker Á, Hágendorn R, Bajor J, Vincze Á, Gyöngyi Z, Mikó A, Csupor D, Hegyi P, Erőss B. Intralesional steroid is beneficial in benign refractory esophageal strictures: A meta-analysis. *World J Gastroenterol* 2018; 24(21): 2311-2319 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i21/2311.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i21.2311

INTRODUCTION

Benign esophageal stricture (BES) is the narrowing of the lumen due to scar formation and fibrosis[1]. The most common, simple strictures need 3-5 sessions of endoscopic dilation at most, while benign refractory esophageal strictures (BRES) require more than 3-5 repeated endoscopic dilation sessions, or it is impossible to achieve a 14 mm wide lumen after 3 sessions of dilation[2].

Patients fail to maintain an effective swallowing action resulting in significant dysphagia. Other symptoms can be atypical chest pain, heartburn and odynophagia. BRES significantly impair the quality of life and may cause severe complications, most importantly weight loss due to malnutrition, but aspiration and regurgitation may occur too[3]. Patients with BRES need regular endoscopic dilations and it is not uncommon that the stricture recurs in days or weeks, necessitating frequent repeat procedures, in some cases multiple times a month.

There are many potential causes of BRES, the most frequent being peptic stricture from pathological acid exposure in gastro-esophageal reflux disease (GERD). Other common causes include radiation, caustic injury, and anastomotic strictures after esophageal surgery or endoscopic submucosal dissection. Less frequent etiologies include eosinophilic esophagitis, congenital and drug-induced stenosis, and it may also develop as a complication of nasogastric intubation or sclerotherapy of esophageal varices[1].

The pathogenesis of BRES is not entirely understood, but chronic inflammation must have a key role. The initial narrowing of the esophageal wall results from edema and muscular spasm as part of an inflammatory process. As the disease progresses, erosions and ulcerations evolve as well as chronic inflammation, leading to fibrous tissue production and collagen deposition. The chronic inflammation probably induces the synthesis of transforming growth factor beta (TGF-β) and α2-macroglobulin, which are inhibitors of collagenase activity. Therefore, depositions of collagen form scars, resulting in the narrowing of the lumen and the rigidity of the wall[3]. Steroids (triamcinolone acetonide injection into 4 quadrants of the stricture[2]) reduce the activity of these inflammatory pathological pathways (*e.g.* the transcription of matrix protein genes, including fibronectin and procollagen), so this may be considered as an effective treatment of scar-forming conditions, providing the basis for the trials included in this meta-analysis[1].

The epidemiology of BRES is not well-known. Most

of the available data are provided by small clinical studies and case studies. The incidence of esophageal stricture seems to be decreasing in parallel with the growing use of proton pump inhibitors (PPIs)[3,4], yet its common cause is GERD and it still occurs in 7%-23% of GERD patients with esophagitis[4].

Endoscopic dilation is an effective standard treatment for BES[1,2]; however, 30%-40% of patients show refractory dysphagia within the first year after intervention and require frequent and repeat dilations in the long term[3]. Several trials have been conducted to determine the efficacy of intralesional steroid injection in the treatment of BRES since the first encouraging results were published in a canine model in 1969[5]. However, a meta-analysis has not been carried out yet.

We wanted to investigate whether intralesional steroid injection in combination with dilation is beneficial in the treatment of BRES.

MATERIALS AND METHODS

A meta-analysis was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[6]. The meta-analysis was registered in advance in PROSPERO under the registration number 42017072329. The PICO items of the search strategy were: Population (P): Patients with esophageal stricture; intervention (I): Dilation plus intralesional steroid injection; control (C): Dilation alone; and outcomes (O): Dysphagia score (DS), total number of repeat dilations (TNRD) and periodic dilation index (PDI).

Search strategy

The article search was carried out in PubMed, Embase and Cochrane databases from inception to 10 April 2017. Two investigators conducted a comprehensive search with a combination of the following keywords: (oesophagus OR esophagus) AND [stricture OR stenosis OR refractory stricture OR benign stricture OR (o)esophageal stricture] AND (dilation OR dilatation) and (steroid OR triamcinolone OR intralesional steroid). No filters were imposed on the searches in the individual databases. References in the primarily eligible articles were screened for additional suitable publications.

Inclusion and exclusion criteria: Articles were selected if they had detailed data on a control (endoscopic dilation only) and a treatment group (endoscopic dilations with intralesional steroid injection). Benign refractory esophageal strictures of all etiologies requiring repeat dilations were included. Language was not an exclusion criterion. Conference abstracts were also included if they contained sufficient data. Case reports, case series, and results from pediatric and non-human trials were excluded. We did not contact the authors of the included articles.

Selection process: Records were managed by the EndNote X7.4 software (Clarivate Analytics, Philadelphia, PA, United States) to remove duplicates. Publications were screened first by title, second by abstract, and finally by full-text, based on our eligibility criteria. The comprehensive search and the selection of the studies were carried out by two investigators.

Data extraction

Numeric and texted data were extracted onto a purpose designed Excel 2016 sheet (Office 365, Microsoft, Redmond, WA, United States). The extracted data were the following: study author, year of publication, geographical location, study design, number of controls and cases, age of the patients, etiology of the strictures, length and location of the stricture, dose of the intralesional steroid injection, the outcomes of the treatment with and without intralesional steroid injection (DS, TNRD and PDI, the complications of the treatment and follow-up time). Data extraction was performed by two investigators and extracted data were checked by a third investigator.

Statistical analysis

In our statistical analysis, we compared the outcomes of treatment with dilation alone to the outcomes of dilation in combination with intralesional steroid injections. Meta-analytical calculations were conducted on the TNRD, PDI and DS. Standardized difference in means (SMD), difference in means (MD) and 95% confidence interval (95%CI) were calculated using the random-effects method developed by DerSimonian and Laird[7]. Results reported in the study in median and range were converted to means and standard deviation with the Hozo method[8]. Heterogeneity among trials was tested with Cochrane's Q and I^2 statistics. According to the Cochrane Handbook, I^2 values of 25%-50%, 50%-75% and > 75% correspond to low, moderate and high degrees of heterogeneity[9]. The Q test implies that the heterogeneity among effect sizes reported in the studies under examination is more diverse than could be explained by random error only. We considered the Q test significant if $P < 0.1$. The presence of any publication bias was examined by visual inspection of the funnel plots.

Assessment of risk of selection and information bias

The assessment of risks of bias and quality was done at the outcome level. The Newcastle-Ottawa Scale[10] was used for case control trials with the following 8 items. Item 1: Were the cases randomly selected subjects with BRES without significant exclusion criteria? Item 2: Were the controls randomly selected subjects with BRES without significant exclusion criteria? Item 3: Was there an endoscopic or radiological diagnosis of BRES? Item 4: Was the diagnosis of non-refractory BES excluded? Item 5: Were the cases and controls comparable? Item

Table 1 Main characteristics of the studies included

Study	Study design	Country	Parameter	Patients		Etiology of BRES	Follow-up (mo)	Complication	
				Cases	Control			Cases	Control
Kochhar *et al*[13] 1999	Crossover	India	PDI	14	14	Mixed	23	1	0
Kochhar *et al*[14] 2002	Crossover	India	PDI	71	71	Mixed	59	0	0
Ahn *et al*[16] 2015	Crossover	New Zealand	PDI	25	25	Mixed	90	0	0
Nijhawan *et al*[16] 2016	Crossover	India	PDI	11	11	Corrosive	18	0	0
Dunne *et al*[17] 1999	RCT	United States	TNRD, DS	20	22	Mixed	60	0	0
Altintas *et al*[18] 2004	RCT	Turkey	TNRD	11	10	Mixed	48	1	1
Orive-Calzada *et al*[20] 2012	Cohort	Spain	TNRD	14	9	Mixed	45	0	1
Hirdes *et al*[19] 2013	RCT	Netherland	TNRD, DS	31	29	Anastomotic	33	5	1
Pereira-Lima *et al*[21] 2015	RCT	Brazil	TNRD, DS	9	10	Mixed	13	0	0
Camargo *et al*[22] 2003	RCT	Brazil	DS	7	7	Mixed	12	0	0
Rupp *et al*[23] 1995	RCT	United States	DS	22	21	Mixed	11	0	0

PDI: Periodic dilation index; NRD: Total number of repeat dilations; DS: Dysphagia score; RCT: Randomized controlled trial; BRES: Benign refractory esophageal stricture.

6: Were the subjects and investigators blinded to the intralesional steroid treatment? Item 7: Was follow-up long enough (≥ 6 mo) for outcomes to occur? Item 8: Was there complete follow up of all subjects enrolled?

For the above detailed items an answer of yes represented low risk, no represented high risk, while lack of description represented unknown risk of bias. Modified NOS was used for studies with cross-over study design with the 7 out of the above detailed 8 items as item 2 regarding the selection of controls was not applicable due to the cross-over study design.

The JADAD scoring system[11] was used for the assessment of randomized controlled trials with the following 5 items. Item 1: Was the study described as randomized? (Yes = 1 point, No = 0 point); Item 2: Was the randomization scheme described and appropriate? (Yes = 1 point, No = -1 point); Item 3: Was the study described as double-blind? (Yes = 1 point, No = 0 point); Item 4: Was the method of double blinding appropriate? (Yes = 1 point, No = -1 point, if the answer of Item 3 was No, Item 4 is not calculable); Item 5: Was there a description of dropouts and withdrawals? (Yes = 1 point, No = 0 point).

Assessment of the grade of evidence

The GRADE system was used to assess the strength of recommendation and quality of evidence of our results. GRADE stands for Grades of Recommendation Assessment, Development, and Evaluation[12].

RESULTS

Results of the selection process

Our search identified 321 articles in Embase, 109 in PubMed, and 12 in the Cochrane database, a total of 11 articles[13-23] (10 in English and 1 in Portuguese) eligible for the quantitative analysis, these included 343 patients in total, 235 cases and 229 controls, as four studies used cross-over design with 121 subjects enrolled. Further 3 articles gave results, but they were not suitable for meta-analytical calculations[24-26]. The selection process is shown on Figure 1 and the main characteristics of the studies included are shown in Table 1.

Results of the statistical analysis

The PDI was comparable in 4 studies with crossover design involving 121 patients[13,14,15,16]. The pooled result showed that PDI significantly decreased in the intralesional steroid plus dilation group, with difference in means method. (MD: -1.16, 95%CI: -1.99, -0.25, P = 0.012). There was a high degree of heterogeneity across the studies included in the analysis for PDI (Q = 11.73, df = 3, P = 0.0084, I^2 = 74.43%). A detailed result of the analysis on PDI by the random effect model is shown in Figure 2.

The TNRD was comparable in 5 studies[17,18,19,20,21], where MD was -1.172 in comparison to the dilation alone group (95%CI: -0.238, 0.053; P = 0.057). The studies in this analysis showed no heterogeneity: (Q = 3.66; df = 4; P = 0.45; I^2 = 0.0%). A detailed result of the analysis on TNRD by the random effect model is shown in Figure 3.

The DS was comparable in 5 studies[17,18,21,22,23], and an improvement could not be observed in the combined therapy group (std. MD: 0.347, 95%CI: -0.383, 1.077, P = 0.351). We note that DS was only comparable with standardization as different studies used different scoring systems. There was a high degree of heterogeneity across the studies included in the analysis for DS (Q = 24.97, df = 4, P < 0.001, I^2 = 83.98%). A detailed result of the analysis on DS by the random effect model is shown in Figure 4.

Complications

Due to the low number of events of complications, statistical analysis was not possible; therefore, only narrative synthesis could be performed. It is important to note that all trials reported low numbers of complications; therefore, this technique seems to be safe. Kochhar *et al*[13] reported transient worsening of dysphagia for 24 h in one patient after the intralesional

Table 2 Results of the Newcastle-Ottawa quality assessment scale for cross-over and cohort studies

	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	
Ahn *et al*[21] 2015	-	N/A	+	+	-	?	+	?	Modified NOS
Kochhar *et al*[21] 2015	-	N/A	+	+	-	?	+	+	Modified NOS
Kochhar *et al*[21] 2015	+	N/A	+	+	-	?	+	+	Modified NOS
Nijhawan *et al*[21] 2015	-	N/A	+	+	-	-	+	+	Modified NOS
Orive-Calza *et al*[21] 2015	-	-	+	?	+	+	+	?	NOS

Item 1: Were the cases randomly selected subjects with BRES without significant exclusion criteria? Item 2: Were the controls randomly selected subjects with BRES without significant exclusion criteria? Item 3: Was there an endoscopic or radiological diagnosis of BRES? Item 4: Was the diagnosis of non-refractory BES excluded? Item 5: Were the cases and controls comparable? Item 6: Were the subjects and investigators blinded to the intralesional steroid treatment? Item 7: Was follow-up long enough (≥ 6 mo) for outcomes to occur? Item 8: Was there complete follow up of all subjects enrolled? For the above detailed items an answer of yes represented low risk, no represented high risk, while lack of description represented unknown risk of bias (- = high risk of bias; ? = unknown or moderate risk of bias; + = low risk of bias). BRES: Benign refractory esophageal stricture; BES: Benign esophageal stricture.

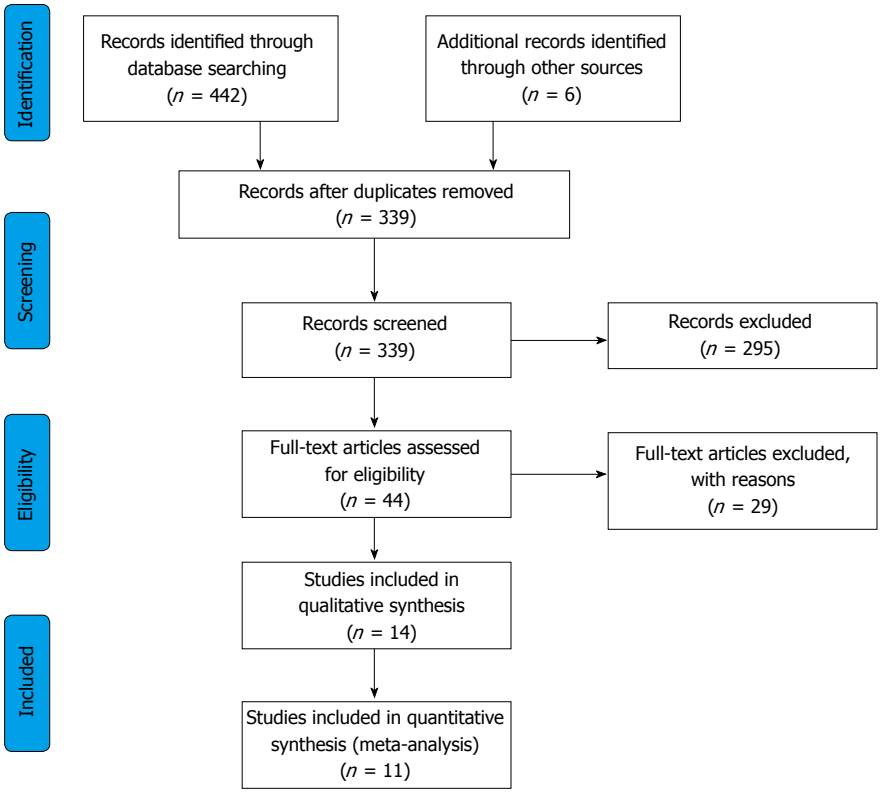

Figure 1 Prisma flow chart of the study selection process.

steroid injection. There were 2 perforations reported by Altintas *et al*[18] one in the dilation only and one in the combined treatment group, both in caustic strictures. Hirdes *et al*[19] reported one gastrointestinal bleeding in the monotherapy group and 5 adverse events, such as 1 laceration and 4 candida esophagitis in the patients treated with intralesional steroid. However, the laceration developed in a patient, who continued the anticoagulant therapy during the procedure, and the other 4 patients received adjuvant chemotherapy, which is a risk factor for candidiasis. One perforation occurred in the dilation only group in Orive-Calzada *et al*[20] trial, with no complication reported in patients with intralesional steroid injection. Other trials did not report any adverse events in either therapy group.

Results of the assessment of risk of bias and quality

Detailed results of the assessments are shown in Table 2 and 3.

DISCUSSION

The summary of our findings are shown in Table 4. Endoscopic dilation as the standard treatment of BES is effective in most cases[1,2], but BRES develops in some cases, necessitating repeated endoscopic dilations in the long term[3]. Endoscopic intralesional steroid injections may be useful and may reduce the number of necessary dilations. However, because of the low incidence of refractory benign esophageal strictures and because of the low number of studies and articles published on the

Table 3 Results of the quality assessment of randomized controlled trials by the JADAD scoring system

	Item 1	Item 2	Item 3	Item 4	Item 5	Overall	Quality
Dunne *et al*[17] 1999	1	-1	0	0	0	0	Low; 0
Altintas *et al*[18] 2004	1	-1	0	0	0	0	Low; 0
Hirdes *et al*[19] 2013	1	1	1	1	1	5	High; 5
Pereira-Lima *et al*[21] 2015	1	1	1	1	1	5	High ,5
Camargo *et al*[21] 2003	1	-1	1	-1	1	1	Low; 1
Rupp *et al*[21] 1995	1	-1	0	0	0	0	Low; 0

Item 1: Was the study described as randomized? (Yes = 1 point, No = 0 point); Item 2: Was the randomization scheme described and appropriate? (Yes = 1 point, No = -1 point); Item 3: Was the study described as double-blind? (Yes = 1 point, No = 0 point); Item 4: Was the method of double blinding appropriate? (Yes = 1 point, No = -1 point, if the answer of Item 3 was No, Item 4 is not calculable); Item 5: Was there a description of dropouts and withdrawals? (Yes = 1 point, No = 0 point). Low range of quality: 3 >, high range of quality: 2 <.

Table 4 Summary of findings

Outcomes	Intervention values	Control values	Number of patients	Quality of evidence (GRADE)	Comments
PDI	0.335/mo	1.355/mo	121	Very low	Only studies with cross-over design were analyzed
	MD: -1.12 95%CI: -1.99 to -0.25 *P* = 0.012				
TNRD	n/a	n/a	165	Very low	Different length of follow up results in high risk of bias
	MD: -1.17 95%CI: -0.24 to 0.05 *P* = 0.057				
DS	n/a	n/a	178	Very low	Different scoring scales were used and different lengths of follow up result in high risk of bias
	SMD: 0.35 95%CI: -0.38 to 1.08 *P* = 0.351				

PDI: Periodic dilation index; TNRD: Total number of repeat dilations; DS: Dysphagia score; MD: Mean difference; SMD: Standardized mean difference.

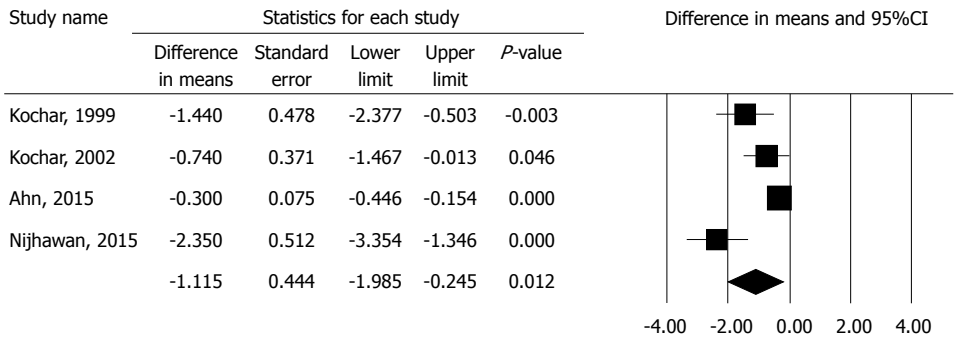

Figure 2 Forest plot of the random effect analysis of the 4 studies concerning periodic dilation index shows a significant decrease of periodic dilation index after intralesional steroid injection in addition to endoscopic dilation.

topic, there is little evidence as to whether this approach is beneficial. Moreover, to the best of our knowledge, no meta-analysis has been carried out yet.

The effectiveness of intralesional steroid injections for BRES was first tested in a canine model in 1969[7]. The first study on humans was carried out by Holder *et al*[27]. They examined 10 pediatric patients, some with post-surgical (anastomotic) strictures and some with corrosive strictures (from acid or lye). They found that additional intralesional steroid treatments were only effective on the anastomotic strictures, but not on the caustic ones.

Among the parameters of the 11 articles included in our meta-analysis, the PDI, TNRD and DS were comparable. It is important to note that all studies used boogie dilators and no studies reported results with balloon dilation

The PDI values were calculated with the mean difference method due to the similar measures and showed a significant improvement of the PDI in the steroid group. These four articles[13-16] examined one patient group, treated first with a series of dilations alone, followed by a dilation combined with intralesional steroid injections afterwards. PDI values were compared before and after the intralesional steroid injections, as these patients all required continuing endoscopic dilation despite the steroid injections. It

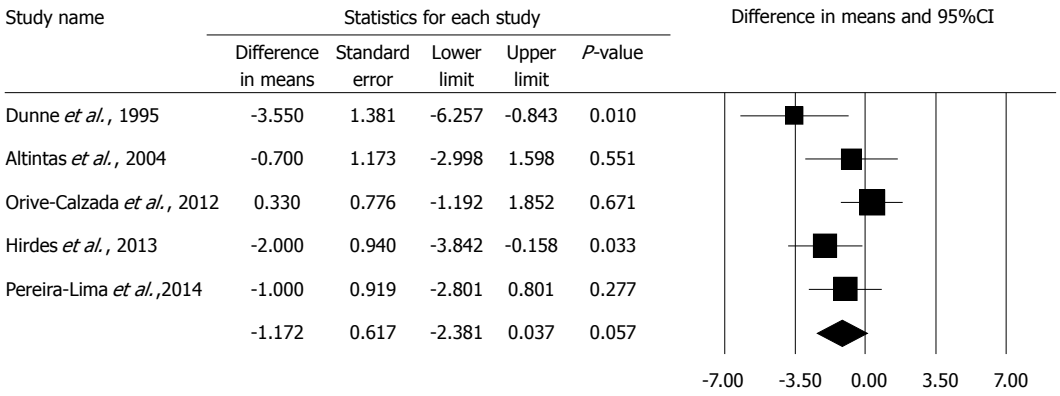

Figure 3 Forest plot of the random effect analysis of the 5 studies concerning total number of repeat dilation shows a non-significant decrease of total number of repeat dilation after intralesional steroid injection in addition to endoscopic dilation.

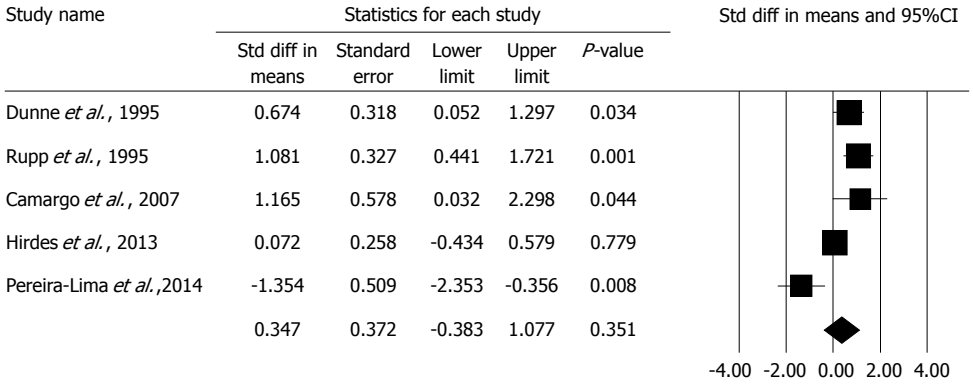

Figure 4 Forest plot of the random effect analysis of the 5 studies concerning dysphagia score shows no significant improvement of dysphagia score after intralesional steroid injection in addition to endoscopic dilation.

must be noted that the study by Nijhawan *et al*[15] showed a statistically significant, strong improvement in the PDI with the combined therapy in patients with corrosive strictures only, so the lack of subgroup analysis results in a high degree of bias.

The TNRD[17,18,19,20,21] was compared with the method noted above. We found a non-significant (P = 0.057) improvement in the combined therapy group using the mean difference method. Interestingly, the article by Orive-Calazda *et al*[20] did not identify improvement compared to the control groups: 9 study group patients and 12 control group patients received 30 and 37 dilations, respectively. The only published multicenter study investigating the TNRD was carried out by Hirdes *et al*[19], but all the patients had an anastomotic stricture, resulting in a bias in the interpretation of their data. In this case, the importance of the subgroup analysis must be highlighted again.

The third parameter, which describes the quality of life best, is the DS. Due to the use of different scoring systems, it was only possible to compare the data from five articles[17,19,21,22,23] with standardization. Based on the statistical analysis of the articles under examination, we did not find any improvement in the steroid group. However, this result cannot be regarded as relevant due to the high heterogeneity of the data. It is important to note that Pereira-Lima *et al*[21], proved a significant improvement in the DS in the combined therapy group in a randomized controlled trial. Hirdes *et al*[19] reported DS results in patients with anastomotic strictures only, which remains a significant bias.

Only a few studies reported outcomes of the treatment with intralesional steroids for different etiologies of the strictures. Kochhar *et al*[13] and Nijhawan *et al*[15] demonstrated significant improvement in caustic strictures. Hirdes *et al*[19] detected no benefit from the combined treatment in anastomotic stricures. Ahn *et al*[16]and Kochhar *et al*[14] showed the most improvement in peptic strictures, both in studies with cross over design.

There was no data on the histological activity of the inflammation of the strictures, although intralesional steroid is likely to be of more benefit in strictures with high degree of active inflammation, than in long standing fibrotic strictures. Subgroup analysis on the degree of inflammation could have given further in depth understanding of the effects of intralesional steroid injections.

Limitations

We observed variable reporting of intervention outcomes. Studies with low patient numbers, heterogeneous data,

use of different scoring systems, and differences in follow-up time resulted in significant difficulties of the analysis. Even though two long-term studies[17,23] were only available as abstracts, they contained the necessary data for the purposes of this meta-analysis. In addition, there was a lack of detailed data on etiological subgroups, which prevented us from performing a subgroup analysis, reulting in a high risk of bias.

In summary, the use of intralesional steroid injections seems to be beneficial in the treatment of BRES with a very low quality of evidence and a weak recommendation. A large, multicenter, prospective randomized trial could provide better evidence for the role of intralesional steroid therapy in the treatment of BRES.

ARTICLE HIGHLIGHTS

Research background

Benign refractory esophageal stricture deteriorates the quality of life, as impaired and often painful swallowing necessitates semi liquid or liquid diet and leads to poor nutrition. Regular endoscopic dilations are a huge burden to the patients, carry risks of complications, require special expertise, and accessories of the endoscopy unit.

Research motivation

Our aim was to investigate if there is any benefit of intralesional steroid injection in addition to endoscopic dilation in the treatment of refractory esophageal strictures.

Research objectives

This is the first comprehensive article in this topic, taking into account all the available evidences and this study quantifies the effect of intralesional steroid injection in addition to endoscopic dilation of benign refractory esophageal stricture.

Research methods

A meta-analysis was performed following the guidelines of the PRISMA P protocol and the review was registered on PROPSPERO. PubMed, Cochrane Library and Embase databases were comprehensively searched for trials eligible for the analysis, describing the outcomes of dilation in comparison to dilation with intralesional steroids. The risks of bias and quality of the individual studies were assessed by using the Newcastle-Ottawa Scale and JADAD Score. The random effect model described by DerSimonian-Laird was used to perform the statistical calculations.

Research results

The statistical analysis involved 343 patients with benign refractory stricture. The results showed that intralesional steroid significantly increased the time between endoscopic dilations, from 1.3-0.3 dilations/month. However, the dysphagia score and the total number of dilation did not improve.

Research conclusions

Intralesional steroid injection increases the time between endoscopic dilations of benign refractory esophageal strictures.

Research perspectives

Further research would be essential to understand the effects of intralesional steroid injection in the treatment of benign refractory esophageal strictures. A multi-center, double blind, randomized controlled trial could give better answers. Detailed data on the outcomes of the treatment in view of the etiology, the time of the diagnosis, the degree of inflammation/fibrosis, the length and location of the stricture should be collected with a long follow up period.

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CASE REPORT

Obeticholic acid for severe bile acid diarrhea with intestinal failure: A case report and review of the literature

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Author contributions: Hvas CL treated the intestinal failure in the patient and wrote the first draft of the manuscript; Ott P managed the communications with Intercept Pharmaceuticals, discussed the treatment and drafted the manuscript; Paine P and Lal S discussed the differential diagnoses during the treatment of the patient and revised the manuscript; Jørgensen SP provided expertise on BAD and FXR biology and revised the manuscript; Dahlerup JF was responsible for treating the patient, handled the communication with National Health Authorities, and revised the manuscript; all authors approved the final version of the manuscript.

Informed consent statement: The patient gave oral and written consent for the publication of this case report. A signed informed consent statement has been uploaded with the submission of the manuscript.

Conflict-of-interest statement: Hvas CL reports lecture fees from Takeda, Ferring, Novartis, MSD, and Tillotts. Ott P reports a lecture fee from Intercept Pharmaceuticals. Lal S reports grants from Fresenius Kabi and Shire. Dahlerup JF reports lecture fees from Pharmacosmos, MSD, and Takeda. All other authors declare that they have no conflicts of interest.

CARE checklist (2013) statement: This case report conforms to the CARE checklist (2013), and a fulfilled PDF version of the checklist was attached with the submission of the manuscript.

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Abstract

Bile acid diarrhea results from excessive amounts of bile acids entering the colon due to hepatic overexcretion of bile acids or bile acid malabsorption in the terminal ileum. The main therapies include bile acid sequestrants, such as colestyramine and colesevelam, which may be given in combination with the opioid receptor agonist loperamide. Some patients are refractory to conventional treatments. We report the use of the farnesoid X receptor agonist obeticholic acid in a patient with refractory bile acid diarrhea and subsequent intestinal failure. A 32-year-old woman with quiescent colonic Crohn's disease and a normal terminal ileum had been diagnosed with severe bile acid malabsorption and complained of watery diarrhea and fatigue. The

diarrhea resulted in hypokalemia and sodium depletion that made her dependent on twice weekly intravenous fluid and electrolyte infusions. Conventional therapies with colestyramine, colesevelam, and loperamide had no effect. Second-line antisecretory therapies with pantoprazole, liraglutide, and octreotide also failed. Third-line treatment with obeticholic acid reduced the number of stools from an average of 13 to an average of 7 per 24 h and improved the patient's quality of life. The fluid and electrolyte balances normalized. The effect was sustained during follow-up for 6 mo with treatment at a daily dosage of 25 mg. The diarrhea worsened shortly after cessation of obeticholic acid. This case report supports the initial report that obeticholic acid may reduce bile acid production and improve symptoms in patients with bile acid diarrhea.

Key words: Bile acid malabsorption; Diarrhea; Farnesoid X-activated receptor; Crohn's disease

Core tip: Bile acid diarrhea develops when excessive amounts of bile acids enter the terminal ileum and exceed the intestinal absorptive capacity. The excess bile acids enter the colon and cause secretory diarrhea. We report a patient with multiple potential causes of chronic diarrhea and suggest a systematic strategy for the diagnosis and treatment of this condition. Furthermore, we describe the use of a new treatment for severe bile acid diarrhea, obeticholic acid, which stimulates the farnesoid X receptor of the terminal ileum and increases fibroblast growth factor 19, thereby decreasing hepatic bile acid production *via* negative feedback.

Hvas CL, Ott P, Paine P, Lal S, Jørgensen SP, Dahlerup JF. Obeticholic acid for severe bile acid diarrhea with intestinal failure: A case report and review of the literature. *World J Gastroenterol* 2018; 24(21): 2320-2326 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i21/2320.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i21.2320

INTRODUCTION

Chronic secretory diarrhea causes intestinal losses of water, sodium, and potassium[1]. In severe cases, it may negatively affect the fluid and electrolyte balance. Chronic secretory diarrhea may be caused by intestinal inflammation, infection, drug side effects or abuse, neuroendocrine tumors, functional diarrhea, or bile acid diarrhea (BAD). When no cause is identified, the condition is termed diarrhea-predominant irritable bowel syndrome (IBS-D)[2].

BAD occurs when excess amounts of bile acids enter the colon and induce colonic fluid and electrolyte secretion and motility changes[3]. Based on the pathophysiology, BAD is classified as type 1, which is caused by ileal resection, disease, or injury, type 2, which consists of primary or idiopathic BAD, and type 3, which is secondary to other conditions, *e.g.*, cholecystectomy[4-6].

The medical treatments of BAD include the bile acid sequestrants colestyramine and colesevelam[3,7,8]. Antisecretory or antimotility drugs such as loperamide and proton pump inhibitors may be added. Some patients with BAD experience an insufficient effect of the available conventional medical treatments and suffer from an impaired quality of life[9,10].

BAD is proposed to result from defective gut-liver feedback mechanisms. Hepatic bile acid synthesis is inhibited by fibroblast growth factor 19 (FGF19) that is produced by ileal enterocytes upon stimulation by bile acids in the terminal ileum *via* the farnesoid X receptor (FXR)[5,11]. Decreased circulating FGF19 levels have been reported in patients with primary BAD[12] and in patients with Crohn's disease and diarrhea[13]. Obeticholic acid, a potent FXR agonist, stimulates ileal FGF19 production and may thereby decrease hepatic bile acid production in BAD[14]. Obeticholic acid is currently used to treat primary biliary cholangitis[15,16] and non-alcoholic steatohepatitis[17,18], but it may also improve BAD[14].

In this case report, we describe the investigations and treatments of a 32-year-old woman with Crohn's disease who suffered from chronic secretory diarrhea that could be potentially attributed to multiple causal factors. Because no infectious, inflammatory, or metabolic cause was demonstrated other than severe bile acid malabsorption, both type 1 and type 2 BAD were considered. The patient experienced a marked and sustained improvement following treatment with obeticholic acid.

CASE REPORT

A 32-year-old Caucasian woman was referred to our unit for refractory diarrhea lasting 10 years. She had a 15-year history of recurrent depression and primary tonic-myoclonic epilepsy. Following the onset of diarrhea, she had been diagnosed with colonic Crohn's disease, and ^{75}selenium homotaurocholic acid test (SeHCAT) scintigraphy[19] performed six years before referral to our unit had revealed a day-7 relative bile acid retention of 0, indicating severe bile acid malabsorption. Conventional treatments for BAD with the bile acid sequestrants colestyramine and colesevelam had a limited or transient effect, and the diarrhea had been unresponsive to antisecretory treatments such as loperamide and codeine phosphate. At the time of referral, the patient received low-dose 6-mercaptopurine for Crohn's disease, 625 mg colesevelam three times per day, 2-8 mg of loperamide per day for BAD, 1500 mg of levetiracetam per day for depression, and 400 mg of lamotrigine per day for epilepsy. The doses of both levetiracetam and lamotrigine had been optimized

Table 1 Potential causes of chronic diarrhea and their diagnostic investigations and results in a patient with severe bile acid diarrhea and intestinal failure

Potential cause of diarrhea	Investigations	Results
Excess bile acid production with deficient retention	SeHCAT scintigraphy	0 retention, indicating an excess loss of bile acids
Active Crohn's disease	Small bowel imaging; colonoscopy; fecal calprotectin	Normal MRI of small bowel and capsule endoscopy; normal colonoscopy with biopsies; fecal calprotectin < 30 mg/kg
Small bowel disease (celiac disease, autoimmune enteropathy)	Duodenal and jejunal biopsies; plasma tissue transglutaminase antibody	Normal biopsies; anti-transglutaminase negative
Clostridium difficile infection	*Clostridium difficile* toxin test	Positive before fecal transplant; negative repeated tests after fecal transplant
Pathogenic intestinal infection	*Salmonella, Shigella, Campylobacter,* and *Yersinia* fecal cultures; PCR for intestinal parasites	Negative
Systemic infection	HIV test; gamma-interferon test for tuberculosis	Negative
Small intestinal bacterial overgrowth (SIBO)	Hydrogen breath test	Negative
Use of antidepressant and antiepileptic medications	Observation during drug holiday; therapeutic drug monitoring	Treatment dose optimized
Laxative use	Urine laxative screen repeated with a patient-blinded sampling time	Negative × 2
Neuroendocrine tumor	Chromogranin A, gastrin, vasoactive intestinal polypeptide, renin, and aldosterone	All within the reference range
Metabolic disease	Thyroid function test and synacthen test	All within the normal range

SeHCAT: Selenium homotaurocholic acid test.

using therapeutic drug monitoring. Prior treatments also included 40 mg of escitalopram per day and 225 mg of venlafaxin per day, which led to poor control of the depression and did not affect bowel function. Anti-inflammatory Crohn's disease treatments with infliximab, adalimumab, natalizumab, and vedolizumab had been provided before referral and did not affect the diarrhea. Crohn's disease remission had been verified *via* a colonoscopy and fecal calprotectin measurement. The duodenal biopsies were normal. The patient had not undergone bowel surgery.

During the first admission to our unit, the results from all investigations were reviewed, and a diagnostic workup was planned (Table 1). The patient's height and weight were 52 kg and 170 cm, respectively. Biochemical analysis revealed severe electrolyte deficiency with low plasma levels of potassium and magnesium. Although the plasma sodium level was normal, sodium depletion was indicated by the urinary sodium being below the detection limit using both single urine measurements and an analysis of a 24-h urine collection. Fecal cultures were negative for *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* species, but a PCR toxin test for *Clostridium difficile* was positive. A 10-d trial of 125 mg of vancomycin four times per day had a transient effect on the diarrhea, and repeat fecal tests were negative. MRI of the small bowel and pan-enteric double balloon endoscopy revealed endoscopic remission, and duodenal, jejunal, ileal, and colonic biopsies were normal. A laxative screen and markers of systemic infection or metabolic disease were normal (Table 1). All medical treatments were reviewed, and because the diarrhea persisted despite conventional treatment, trials of spironolactone, octreotide, and liraglutide were initiated during the admission but were without effect or produced unacceptable side effects (Table 2). The dose of 6-mercaptopurine was optimized using thiopurine metabolite measurements, revealing a normal TPMT genotype and phenotype, an E-TGN level of 247 nmol/mmol HGB, and an E-MeMP level of 2354 nmol/mmol HGB.

Due to having persistent dehydration with a passage of up to 5 L of watery stools per day, the patient was considered for long-term intravenous support. The patient's potassium levels normalized upon infusions of up to 100 mmol of potassium per day, but the urinary sodium became measurable in the 24-h urine samples only after hypertonic NaCl was applied at 2000 mL of 3% NaCl per day. Because intravenous supplementation was necessary to sustain a normal hydration and electrolyte status, the patient was classified as having type III intestinal failure, subtype A3[20]. A scheduled regimen was established with twice weekly infusions of fluids and electrolytes, but the patient remained underweight, had watery diarrhea, and had a poor quality of life.

A trial of obeticholic acid was considered because of the promising initial reports[14]. Following collaboration with Intercept Pharmaceuticals and approval from the National Health Authorities, we were able to start obeticholic acid during admission. In the initial investigation, a repeat *Clostridium difficile* test was positive, and a fecal transplant using an anonymous donor was performed following a short vancomycin taper. Subsequently, the mean number of bowel passages per 24 h decreased from a mean of 17 to a mean of 13. Obeticholic acid was then started at 10 mg per day and increased to 25 mg per day after 4 d. Importantly, obeticholic acid further reduced the number of bowel movements from a mean of 13 to a mean of 7 per 24 h (Figure 1). When the number of bowel movements

Table 2 Anti-diarrheal drug treatments, their mechanisms of action, and their treatment results in a patient with severe bile acid diarrhea and intestinal failure

Drug	Mechanism of action	Treatment effect
Colestyramine (Questran®)	Bile salt sequestrant	Limited effect
Colesevelam (Cholestagel®)	Bile salt sequestrant	Limited effect
Pantoprazole	Proton pump inhibitor	No effect
Loperamide (Imodium®)	Decreases intestinal motility	No effect
Codeine phosphate	Decreases intestinal motility	No effect; sedation
Spironolactone	Increases renal potassium reabsorption	No effect on potassium deficiency
Octreotide	Antisecretory	No effect; abdominal pain
Liraglutide (Victoza®)	Increases glucagon-like peptide 1 (GLP-1)	No effect; weight loss of 2 kg to 52 kg
Obeticholic acid (Ocaliva®)	Stimulates ileal FGF19 production, thereby inhibiting hepatic bile acid production	Marked reduction of stool volume and fecal electrolyte loss

FGF19: Fibroblast growth factor 19.

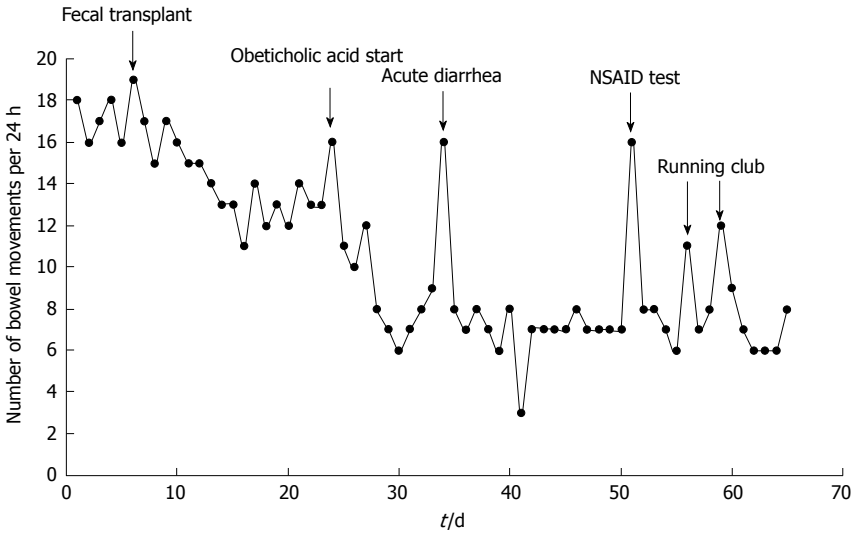

Figure 1 Bowel movement frequencies before and during the initial two months of treatment with 25 mg obeticholic acid once daily for severe bile acid diarrhea with intestinal failure.

during the two weeks of treatment with 25 mg of obeticholic acid per day was compared with that of the two weeks before treatment, the difference was highly statistically significant (P = 0.00001, Mann-Whitney U test). While nightly bowel movements had been a persistent problem before the initiation of treatment, these were reduced from a mean of 3 nightly bowel openings to a mean of 2 nightly bowel openings following treatment, and on occasional nights, the patient did not open her bowel during the night. The patient's weight increased by 2 kg to 54 kg, and she was weaned off intravenous fluid support. She resumed social activities, including running, although this occasionally induced an increase in the number of bowel movements (Figure 1). She remained sensitive to non-steroid anti-inflammatory drug treatment because a single dose of 400 mg of ibuprofen transiently induced liquid stools (Figure 1). The quality of life was estimated using the Euroqol EQ-5D-3L questionnaire (https://euroqol.org). Before the treatment, the patient reported an overall wellbeing of 35 on a 0-100 scale. This increased to 85 following two weeks of obeticholic acid treatment and remained at this level for six months of follow-up.

To examine whether the effects were specific to obeticholic acid and whether the effect would last without continued treatment, the patient agreed to a treatment pause. Following three days without obeticholic acid, the patient's condition deteriorated, with an increase in the number of bowel movements in 24 h from 7 to 16 and profound hypokalemia. Shortly after restarting obeticholic acid, the patient's bowel control was reestablished. During 6 mo of follow-up, we observed no adverse effects, and control of Crohn's disease, epilepsy, and depression did not change. A single episode of increased serum pancreatic amylase (266 U/L; reference range: 10-65 U/L) necessitated a pause of the 6-mercaptopurine treatment. Ultrasound examination revealed a normal pancreas and bile ducts, and the p-amylase level normalized. A diagnosis of acute pancreatitis could therefore not be confirmed, and treatment was restarted without further episodes or an increase in the pancreatic or liver function tests. The plasma lipids were slightly elevated before the treatment and decreased during the obeticholic

acid treatment. Thus, the patient's total cholesterol decreased from 7.5 to 5.9 mmol/L, and her LDL-cholesterol level decreased from 4.5 to 3.1 mmol/L, while her HDL-cholesterol increased slightly from 2.0 to 2.1 mmol/L. Measurements of fasting serum FGF19 were performed once before and six times during treatment with obeticholic acid, using the Human FGF-19 Quantikine ELISA kit DF 1900 (R&D Systems, Minneapolis, MN, United States). Although the mean FGF19 level increased from 35.7 to 167.0 pg/mL during treatment with 25 mg per day, we observed a marked fluctuation in the serum FGF levels during obeticholic treatment, with serum FGF19 concentrations ranging from 21 pg/mL to 728 mg/mL.

DISCUSSION

This case report demonstrates the challenges related to the diagnosis and treatment of patients with multifactorial chronic diarrhea. In this patient, a thorough and systematic evaluation of several differential diagnoses was pivotal for understanding the causes of chronic diarrhea in the presence of a severely disrupted electrolyte balance and intestinal failure. Because the SeHCAT retention rate was 0 on day 7, an overproduction of bile acids in combination with severe bile acid malabsorption was indicated. In the absence of other causes of chronic diarrhea, we concluded that the patient had severe BAD. Before treatment with obeticholic acid, the patient had intestinal failure with a dependency on intravenous fluid and electrolyte support. To the best of our knowledge, this is the first report of BAD of such severity.

For patients with chronic diarrhea, the SeHCAT scintigraphy identifies those with BAD and, hence, a treatable cause of diarrhea[8,19,21-23]. It further helps to tailor the treatment. This investigation therefore remains an important tool in the diagnostic workup and should be considered in patients with Crohn's disease and unresolved diarrhea[6].

While this patient was refractory to conventional therapies for diarrhea, she improved both clinically and biochemically following treatment with obeticholic acid. This adds to the promising data that indicate obeticholic acid may improve BAD *via* a modulation of negative feedback signaling of FGF19 on hepatic bile acid production[14]. Obeticholic acid is marketed for the treatment of primary biliary cholangitis and has been investigated in dosages of 10 mg to 50 mg daily for 3 mo[15] and up to 10 mg daily for 12 mo[16]. Pruritus was the most common side effect and occurred in up to two-thirds of the treated patients, even at low doses. We observed no side effects in this patient. Because the treatment was well-tolerated, and the improvements of fluid balance and quality of life were sustained during the follow-up, we did not change the treatment dose.

We measured fasting serum FGF19 levels both before and during treatment and found that obeticholic acid increased FGF19 levels, but with substantial variation between samples obtained during treatment. The finding supports that hepatic bile production is inhibited by FGF19 signaling following the obeticholic acid-induced stimulation of FXR in ileal enterocytes[12,24,25]. It also emphasizes that the use of FGF19 measurement should be validated. In general, FGF19 levels depend on renal function, age, and systemic inflammation[26,27]. In patients with Crohn's disease, FGF19 levels are generally lower than in control patients, and low levels are associated with ileal resection and with active disease, independently of ileal resection[13].

In conclusion, we found that treatment with oral obeticholic acid (25 mg daily) induced a marked and sustained improvement of bowel function, fluid and electrolyte balance, and quality of life in this patient with severe BAD and intestinal failure. Future clinical trials should investigate the long-term clinical effects of obeticholic acid, including safety measures and serum FGF19 dynamics.

ARTICLE HIGHLIGHTS

Case characteristics

A 32-year-old woman with chronic diarrhea that had multiple potential causes including bile acid diarrhea, Crohn's disease, and medications for epilepsy and depression.

Clinical diagnosis

Bile acid diarrhea (BAD), diagnosed by selenium homotaurocholic acid test scintigraphy with 0 bile acid retention after seven days.

Laboratory diagnosis

Persistently low plasma levels of sodium and potassium and undetectable 24-h urine sodium excretion, indicating intestinal failure with dependency of intravenous fluid support.

Pathological diagnosis

Normal duodenal, jejunal, ileal, and colonic biopsies, indicating quiescent Crohn's disease. Positive *Clostridium difficile* toxin PCR test indicating *Clostridium difficile* colitis.

Treatment

Clostridium difficile colitis was treated with vancomycin followed by fecal microbiota transplantation. Bile acid diarrhea was refractory to conventional treatments including colestyramine and colesevelam, and oral obeticholic acid treatment was commenced at 10 mg per day, increasing to 25 mg per day. Upon this, the patient's bowel habits and quality of life improved.

Related reports

Obeticholic is licensed for primary biliary cholangitis and has been used in non-alcoholic steatohepatitis. It was recently reported that obeticholic acid may improve bile acid diarrhea through induction of fibroblast growth factor 19 that inhibits hepatic bile production.

Term explanation

BAD–bile acid diarrhea, resulting from excess hepatic production and/or deficient ileal reabsorption of bile acids, which in turn induces colonic fluid and electrolyte secretion and leads to chronic secretory diarrhea.

Experiences and lessons

In patients with chronic diarrhea, a thorough and systematic diagnostic workup

may help to differentiate between potential causes of diarrhea. Some patients with bile acid diarrhea are refractory to conventional treatments. Obeticholic acid may be of clinical benefit in these patients.

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